Active Packaging Applications for Food

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ABSTRACT

The traditional role of food packaging is continuing to evolve in response to changing market needs. Current drivers such as consumer's demand for safer, "healthier," and higher-quality foods, ideally with a long shelf-life; the demand for convenient and transparent packaging, and the preference for more sustainable packaging materials, have led to the development of new packaging technologies, such as active packaging (AP). As defined in the European regulation (EC) No 450/2009, AP systems are designed to "*deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food.*" Active packaging materials are thereby "*intended to extend the shelf-life or to maintain or improve the condition of packaged food*". Although extensive research on AP technologies is being undertaken, many of these technologies have not yet been implemented successfully in commercial food packaging systems. Broad communication of their benefits in food product applications will facilitate the successful development and market introduction. In this review, an overview of AP technologies, such as antimicrobial, antioxidant or carbon dioxide-releasing systems, and systems absorbing oxygen, moisture or ethylene, is provided, and, in particular, scientific publications illustrating the benefits of such technologies for specific food products are reviewed. Furthermore, the challenges in applying such AP technologies to food systems and the anticipated direction of future developments are discussed. This review will provide food and packaging scientists with a thorough understanding of the benefits of AP technologies when applied to specific foods and hence can assist in accelerating commercial adoption.

Keywords: active packaging, oxygen scavenger, antimicrobial packaging, antioxidant releaser, ethylene absorber.

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Nomenclature

AA	ascorbic acid
AITC	allyl isothiocyanate
AnV	*p*-anisidine value
AP	active packaging
BEO	basil leaf essential oil
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
BI	browning index
CEO	cinnamon essential oil
CFU	colony-forming units
CPP	cast polypropylene
CSP	catalytic system with palladium
EDTA	ethylenediaminetetraacetic acid
EO	essential oil
EVOH	ethylene vinyl alcohol
EFSA	European Food Safety Authority
FFA	free fatty acids
GC	gas chromatography
GRAS	generally regarded as safe
HDPE	high-density polyethylene
IC	inhibitory concentration
IU	international units

89	LAB	lactic acid bacteria
90	LAE	ethyl-N$^{\alpha}$-dodecanoyl-*L*-arginate or lauric arginate ester
91	LDPE	low-density polyethylene
92	LLDPE	linear low-density polyethylene
93	MA	modified atmosphere
94	MAP	modified atmosphere packaging
95	MDA	malonaldehyde
96	MO	microorganism
97	MRE	meal-ready-to-eat
98	OPET	oriented polyester
99	OPP	oriented polypropylene
100	OS	oxygen scavenger
101	OTR	oxygen transmission rate
102	PBAT	poly(butylenadipate terephthalate)
103	PCL	polycaprolactone
104	PE	polyethylene
105	PET	poly(ethylene terephthalate)
106	PFO	polyfuryloxirane
107	PLA	polylactic acid
108	PP	polypropylene
109	PS	polystyrene
110	PV	peroxide value
111	PVC	poly(vinyl chloride)

PVDC	poly(vinylidene chloride)
RH	relative humidity
SAP	super-absorbent polymer
SiO_x	silicon oxide
SP/IP	smart and intelligent packaging
TBA	thiobarbituric acid
TBARS	thiobarbituric acid-reactive substances
TCC	total coliform counts
TiO_2	titanium dioxide
TMA	trimethylamine
TVC	total viable counts
(U.S.)FDA	(United States) Food and Drug Administration
WVTR	water vapor transmission rate
ZnO	zinc oxide

Introduction

Packaging plays critical role in the food supply chain. The primary function of packaging is to serve as a container for the food enabling efficient transport within the whole supply chain, preventing any physical damage, and protecting against manipulation and theft. Packaging also meets the fundamental need to maintain food quality and safety from production to final consumption by preventing any unwanted chemical and biological changes. Hence, the packaging acts as a barrier to protect the food from environmental influences such as oxygen, moisture, light, dust, pests, volatiles, and both chemical and microbiological contamination (Coles and others 2003; Yildirim 2011; Arvanitoyannis and Oikonomou 2012; Pereira de Abreu and others 2012). The protective role of the packaging is primarily passive, acting as a barrier between the food, the atmosphere surrounding the food, and the external environment. However, there are some exceptions, such as fresh produce, for which highly gas permeable or perforated packaging materials are used to allow gas exchange through the packaging (Lee and Paik 1997; Hussein and others 2015). Such packaging systems, however, are limited in their ability to further extend the shelf-life of the packaged food. Over recent decades, consumer concern about the safety and additive content of food has received much attention. There is an increasing trend to natural high-quality foods, which are non-processed or minimally processed, do not contain preservatives, but offer an acceptable shelf-life (Singh and others 2011; Gerez and others 2013). In response, the protective function of packaging has been refined and improved leading to the development of new packaging technologies, such as modified atmosphere packaging (MAP) (Ohlsson and Bengtsson 2002; Rodriguez-Aguilera and Oliveira 2009;

Sandhya 2010; Cooksey 2014; Zhuang and others 2014), active packaging (AP) (Singh and others 2011; Yildirim 2011; Arvanitoyannis and Oikonomou 2012; Pereira de Abreu and others 2012; Dobrucka and Cierpiszewski 2014; Realini and Marcos 2014; Kuorwel and others 2015; Brockgreitens and Abbas 2016), smart and intelligent packaging (SP/IP) (Kerry and Butler 2008; Lee and Rahman 2014; Realini and Marcos 2014; Biji and others 2015; Brockgreitens and Abbas 2016), and the application of nanomaterials (Imran and others 2010, Llorens and others 2012, Rhim and others 2013, Reig and others 2014, Rhim and Kim 2014, Bumbudsanpharoke and others 2015). The emphasis of this review is on active packaging.

Active packaging is an innovative approach to maintain or prolong the shelf-life of food products while ensuring their quality, safety, and integrity. As defined in the European regulation (EC) No 450/2009, active packaging comprises packaging systems that interact with the food in such a way as to "*deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food*" (European Commission 2009). Active packaging systems can be divided into active scavenging systems (absorbers) and active-releasing systems (emitters). Whereas the former remove undesired compounds from the food or its environment, for example, moisture, carbon dioxide, oxygen, ethylene, or odor, the latter add compounds to the packaged food or into the headspace, such as antimicrobial compounds, carbon dioxide, antioxidants, flavors, ethylene or ethanol. Table 1 provides an overview of the primary active packaging technologies and their potential benefits in food applications.

The addition of active substances, such as antimicrobials and antioxidants, through the use of AP instead of direct addition to the food, may decrease the amount of such substances required. Traditionally, active substances are added to the bulk of the food, whereas for most fresh and processed food, food degradation or microbial growth occurs at the surface of the food. Furthermore, the activity of the active substances when directly added to food may be reduced or inhibited as a result of interaction between the active substances and the food components, and/or during the processing of the food. Therefore, the addition of active substances via active packaging may be more effective than their addition to the bulk of the food.

A large variety of active packaging systems has been developed and, to date, numerous reviews have emphasized the potential of active packaging technologies to supply safer, "healthier", and higher-quality foods to the consumer (Kuorwel and others 2015; Brockgreitens and Abbas 2016). However, the number of reviews presenting the benefits of active packaging technologies applied to specific food products is limited (Llorens and others 2012; Pereira de Abreu and others 2012; Cichello 2015). Active packaging technologies that have been evaluated in model systems may not always behave in the same way in real food applications. The complex structure of the food may influence the activity of the packaging. For example, the release rates, absorption rates, or diffusion rates of active substances may be affected. Moreover, active substances or carriers may react with food components or bind to them, thereby inhibiting the desired activity. It is therefore important to critically review active packaging studies pertaining to specific foods thereby enabling food and

packaging scientists to better understand the benefits of such systems and potentially accelerate the adoption of the technologies in commercial applications.

The primary focus of this review is to provide an overview of those active packaging technologies that have already been successfully applied to food, thereby highlighting the benefits for the particular food products. Specific emphasis is given to antimicrobial and antioxidant packaging systems. Furthermore, packaging systems that emit carbon dioxide or absorb oxygen, moisture or ethylene, and have been successfully implemented are discussed in depth.

Oxygen Scavengers

The application of oxygen scavengers (OS) is one of the main active packaging technologies that aims to remove any residual oxygen present in the food package (Solovyov 2010; Arvanitoyannis and Oikonomou 2012; Realini and Marcos 2014) or improve barrier properties by acting as an active barrier (Sängerlaub and others 2013a). Several food products are sensitive to oxygen, thus, the food industry seeks to exclude oxygen from food packaging. This is mainly achieved using gas flushing or modified atmosphere packaging processes. However, the residual oxygen-concentration in the package often remains between 0.5-5% (Solovyov 2010; Gibis and Rieblinger 2011; Pereira de Abreu and others 2012) and may further increase during storage. This can be due to insufficient evacuation during the packaging process, oxygen permeation through the packaging material or poor sealing (Pereira de Abreu and others 2012), or due to oxygen dissolved in the food itself

being released into the headspace of the package to reach equilibrium with the gas phase (Pénicaud and others 2012).

Oxygen in packaging negatively affects the quality and shelf-life of several foods as it leads to oxidation of the product (Choe and Min 2006) or promotes the growth of aerobic microorganisms (Lee 2010; Solovyov 2010) resulting in color modifications (Møller and others 2000; Nannerup and others 2004; Larsen and others 2006; Gibis and Rieblinger 2011; Hutter and others 2016), or sensory changes (Jacobsen 1999; Granda-Restrepo and others 2009a; Li and others 2013), or nutritional losses (Chung and others 2004; Lopez-Gomez and Ros-Chumillas 2010; Van Bree and others 2012). A reduction in the residual oxygen level of a food packaging can be achieved through the application of oxygen scavengers, in some cases down to <0.01 vol.-%. The oxygen-scavenging mechanism is mostly chemical. Most common are iron-based scavengers (Miltz and Perry 2005; Galotto and others 2009; Braga and others 2010; Gibis and Rieblinger 2011; Polyakov and Miltz 2016), of which the OS activity is triggered by moisture so that the reduced iron is irreversibly oxidized to a stable ferric oxide trihydrate complex (Solovyov 2010). In contrast, other applied metals, such as cobalt (Galdi and others 2008; Damaj and others 2014), act as a catalyst for the oxidation of polymers, or palladium (Yildirim and others 2015), that catalyzes the oxidation of hydrogen into water. Other systems that scavenge oxygen chemically include photosensitive dyes (Maloba and others 1996; Miller and others 2003; Zerdin and others 2003; Perkins and others 2007), ascorbic acid (Matche and others 2011; Pereira de Abreu and others 2012), gallic acid (Wanner 2010; Ahn and others 2016), or unsaturated fatty acids (Arvanitoyannis and Oikonomou 2012; Pereira de Abreu and others 2012). Moreover, there are also biochemical

mechanisms that work through the use of enzymes (Andersson and others 2002; Fernández and others 2008; Kothapalli and others 2008; Nestorson and others 2008; Gohil and Wysock 2014), or biological approaches using bacterial spores (Anthierens and others 2011), or yeasts that are immobilized in a solid material (Edens and others 1992). Some commercially available oxygen-scavenging solutions are SHELFPLUS® O2 (OS-masterbatch, Albis Plastic GmbH, DE), AMOSORB™ ColorMatrix™ (different OS-solutions, PolyOne™, Europe Ltd., UK), Cryovac® (OS-film, Sealed Air Corporation, USA), AGELESS OMAC® (OS-film, Mitsubishi Gas Chemical Inc., USA), OxyRx® (OS-containers, Mullinix Packages Inc., USA), or Aegis® OXCE (OS-masterbatch, Honeywell International Inc., USA).

Much published work about oxygen-scavenging technologies suggests they have great potential in food applications. However, such research has mainly been performed using oxygen-scavenging sachets containing iron powder (Charles and others 2003; Solovyov 2010; Antunez and others 2012; Cruz and others 2012; Kartal and others 2012; Chounou and others 2013; Cichello 2015). In contrast to Asia or the USA, sachet-based applications are not well accepted by consumers in European countries (Restuccia and others 2010), as they are recognized as foreign bodies in food containers. In fact, the risk of accidental breakage, which can lead to involuntary consumption of the content, is only one of the disadvantages of such sachet-based active packaging technologies. Further drawbacks include the requirement of an additional packaging operation step or their unsuitability in combination with beverages or moist foods due to moisture sensitivity (Suppakul and others 2003; Day 2008; Pereira de Abreu and others 2012). Alternatively, several new oxygen-scavenging technologies have been developed over the last decade, such as incorporating active

263 substances directly into packaging films or containers. However, only few of them have been
264 successfully implemented in real food systems. Consequently, studies demonstrating the
265 benefits of alternative oxygen-scavenging systems to particular food products are rather
266 rare. Table 2 provides an overview of several oxygen-scavenging technologies which have
267 already been applied to food products.
268
269 **Iron.** Shin and others (2009) applied an iron-based OS packaging to extend the shelf-life of
270 processed meat products. Meatballs were packed in active PP-based multilayer trays
271 containing OS materials (40, 80 and 100% w/w) in the middle layer. During a storage time of
272 up to 9 months at 23 and 30 °C, oxidative-induced color and flavor changes of the meatballs
273 packaged in the active OS containers (100% w/w) were significantly lower compared to
274 those in the passive packages. This was also confirmed by TBA values (thiobarbituric acid)
275 indicating less lipid peroxidation of meatballs in these OS packages.
276
277 Military rations constitute a range of products in which a long shelf-life is of particular
278 interest. These food products are critical because the military requires good stability for a
279 minimum of 3 years without refrigeration (Gomes and others 2009) which presents a
280 challenge, especially with components with high oil content that are susceptible to oxidative
281 deterioration. Within this context, Gomes and others (2009) investigated the influence of an
282 iron-based OS-containing laminate material for its ability to extend the shelf-life of a hot-
283 filled meal-ready-to-eat (MRE) cheese spread, a component of military operational rations.
284 The authors demonstrated that the proposed O_2-absorbing laminate reduced the initial
285 headspace oxygen concentration in MRE pouches from 20.4 to 6.82 vol.-% (67.44 vol.-%

decrease) within the first 24 h, was further reduced to below 1 vol.-% within 11 days of storage, and it remained below this level throughout the whole storage period (1 year). The oxygen concentration in the regular MRE pouches also decreased by 50% during the first 15 days and remained constant at a concentration of 5 vol.-%. This oxygen decrease, however, was assigned to the oxidative degrading reactions of the food. After 1 year of storage, the positive effect of the O_2-absorbing laminate was illustrated through MRE cheese spread with significantly lower rancidity and higher sensory acceptance compared to MRE stored in packages without OS. Furthermore, the vitamin C content of the samples in the O_2-absorbing laminate could be better preserved resulting in an almost 1.5 times higher vitamin C content compared to the control samples. Thus, the authors clearly demonstrated that the O_2-absorbing laminate removed oxygen before it was otherwise available for degrading reactions in the food product.

A novel iron-based oxygen scavenger using iron nanoparticles, blended with activated carbon, sodium chloride, and calcium chloride, was produced and evaluated by Mu and others (2013). Nano-sized iron-based oxygen scavenger (110 nm average particle size) exhibited a higher oxygen-scavenging rate (estimated 13.5 mL/d) compared to the oxygen scavenger-containing microscale pure iron powder (about 20 µm; estimated 1.8 mL/d). Moreover, the scavenger capacity of the nano-sized scavenger was found to be almost 1.4 times higher than that of its micro-sized counterpart. This advantage was successfully utilized to inhibit lipid oxidation in lipid-containing foods. Storage of roasted sunflower seeds and walnuts over a period of 120 days showed that the iron nanoparticles were an effective means of inhibiting lipid oxidation. While the peroxide values (PV) of the control samples

without OS increased (sunflower seeds: from 4.32 to 46.89 meq O_2/kg oil, walnuts: from 2.41 to 21.85 meq O_2/kg), the PV of the samples containing the nano-sized oxygen scavenger were shown to be significantly lower (sunflower seeds: 19.82 meq O_2/kg, walnuts: 8.84 meq O_2/kg) after 120 days of storage. Similar results were obtained for secondary lipid oxidation. Although significant differences in the *p*-anisidine values (AnV) between the samples were only observed after 40 and 80 days of storage of sunflower seed and walnut samples, respectively; the AnV of the samples with the scavenger were about half that without OS after 120 days. Nevertheless, the use of nanosized iron powder may further reduce consumer acceptance due to possible leakage and unintentional consumption.

In some cases, preservation of food quality can be affected even if high barrier and modified atmosphere packaging are applied, for example, if the sealing layer of packages exhibits defects, particularly when these defects have a critical size that is below the detection limit of standard leak testers of 10 µm (Sasaki and Kamimura 1997; Sängerlaub and others 2013a). In this context, Sängerlaub and others (2013a) simulated food packages with pinhole defect sizes of 10 µm. They performed long-term storage experiments (300 days) to compare O_2 absorption with a snack food product, salami in a baked bread roll, in packages with and without an iron-based multilayer OS film. Although reactions of the food with oxygen could not be fully prevented, oxidation reactions were significantly reduced by the application of the OS film. Salami samples packed with OS showed less difference in color (ΔE of 5.4) compared to those packed without OS (ΔE of 6.9). Additionally, the use of OS film led to less lipid oxidation in the product resulting in hexanal concentrations almost 4 times lower than those in the control samples without OS. Hence, the applicability of an oxygen

scavenger layer in the barrier film structures to provide extended protection against oxygen penetration through such seal defects was confirmed.

Ascorbic Acid. Matche and others (2011) modified LLDPE films by blending them with iron and ascorbic acid (Fe/AA) or zinc and ascorbic acid (Zn/AA). Thereby, the ascorbic acid (AA) acted as a reducing agent and the transition metals were used to catalyze the oxidation reaction (Graf 1994). The incorporation of these reactive chemicals led to active films with an OS performance of 47.6 and 37.4 mL, respectively, in 750 hours. With the application of the OS films in the form of sealed bags to bakery products, an overall shelf-life extension of packaged buns and bread slices could be demonstrated. In particular, the study of Matche and others (2011) showed that microbial growth was retarded from 2 to 5 days in samples packaged with the OS film. Instrumental texture analysis and moisture analysis revealed that both bun and bread samples without OS film were significantly firmer and dryer, respectively, after 4 days. This was explained by the lower water vapor transmission rates (WVTR) of both Fe/AA (17.2) as well as Zn/AA films (17.4) compared to the pure LLDPE film (20 g/m^2 100 gauge/day), which was used as a control. Moreover, both modified variations showed lower oxygen transmission rates (OTR). Sensory testing additionally supported the obtained measured data as the bread slices and buns packed in OS films were sensorially acceptable (softness and taste) up to 5 and 6 days, respectively, whereas the control samples were not acceptable anymore on the second day. Despite these positive results, the study lacks information about the package size and volume as well as the evolution of the headspace oxygen concentration of the packaged bakery products. This hinders the

understanding of the capacity of the scavengers and the correlation between the OS activity and the extension of the mold-free shelf life of the product.

Photosensitive dyes. Maloba and others (1996) used a photosensitive OS film to improve oxidative stability of sunflower oil. The applied ethyl cellulose polymer film contained the common organic dyes eosin and curcumin and the synthesized polyether polyfuryloxirane (PFO). When exposed to light, this OS film uses energy transfer to convert triplet oxygen ($^3O_2$) into highly reactive singlet oxygen ($^1O_2$), which is absorbed irreversibly by the PFO. The method and mechanism behind such systems are explained by Rooney (1995). The sunflower oil was stored in the presence of the OS film and oxidative stability was evaluated by determination of peroxide values (PV) and gas-chromatographic (GC) measurement of headspace hexanal. To evaluate the influence of illumination, an initial irradiation period of 2 days at 2000 lux was followed by continuous illumination at normal room light (500 lux), the latter imitating the light level commonly encountered on retailer's shelves. Control samples were additionally stored under light exclusion. It was shown that sunflower oil stored under illumination, at 23 °C and 12 weeks of storage, in the presence of the OS film exhibited significantly higher oxidative stability compared to all control samples. PVs were significantly lower with about 20 meq/kg (OS film, illuminated) compared to about 65 meq/kg (no OS film, dark), about 75 meq/kg (no OS film, illuminated + antioxidant) and about 100 meq/kg for the sunflower oil alone (illuminated). The same trend was observed for the amount of headspace hexanal indicating a high correlation ($r > 0.95$) between the 2 methods for measuring rancidity. Thus, the OS film showed the potential to be applied with a wide range of foods that contain polyunsaturated oils and that are stored under illumination at the

point of sale for long periods. However, industrial application of such an OS system might be challenging, as the initial irradiation has to be optimized to ensure that the oxygen concentration in the headspace is reduced below the critical oxygen concentrations to the specific food products.

A similar OS film has also been successfully implemented with orange juice to protect juice from oxidative degradation. Zerdin and others (2003) performed a storage study (1 year) with orange juice filled in vacuum-sealed OS pouches. The OS activity of these pouches was triggered by UV illumination just prior to packaging. The authors demonstrated that at 25 and 4 °C, the initial dissolved oxygen concentration of 2.7 ppm in the orange juice samples packed in OS pouches was reduced to below 0.04 ppm within 3 and 7 days, respectively. In contrast, for the control pouches without OS, dissolved oxygen in the orange juice was above 0.04 ppm up to at least 35 and 77 days, respectively, clearly reflecting the impact of the competition for the oxygen between the OS film and the ascorbic acid as well as the impact of temperature. This was confirmed by the correlating ascorbic acid retention which was significantly higher for the samples using the OS pouches with 30.0% (25 °C) and 73.2% (4 °C) compared to the control pouches showing an ascorbic acid retention of 7.29 (25 °C) and 51.3% (4 °C) after one year of storage. Furthermore, it was shown that the loss in ascorbic acid also correlated with an increase in the non-enzymatic browning of the juice. Samples in the OS pouches stored at 4 °C had a browning index below 0.15 during the entire storage period, indicating freshly pressed orange juice (Johnson and others 1995). Storage at 25 °C led to an increased browning, however, the browning index of samples in the OS pouches was significantly lower with about 0.34 compared to that of the control which was

about 0.44. Hence, the rapid removal of oxygen was found to be an important factor in sustaining a higher concentration of ascorbic acid and color preservation in orange juice over long storage. Inclusion of such an OS film to juice packaging might enable juice producers to reduce or omit the use of antioxidant substances, however, the additional step of UV illumination on the production line needs to be taken into consideration.

In dairy products such as probiotic yogurt, the application of oxygen scavengers can be of particular interest since dissolved oxygen has a negative effect on the viability of probiotic bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium* spp. which are essential for yogurt production (Shah and others 1995). To control the amount of dissolved oxygen, Miller and others (2003) applied an OS film containing a reducible organic compound such as a substituted anthraquinone (Rooney 1999). Unlike the OS film of Maloba and others (1996), this film did not require a constant source of light. It only required UV light exposure to trigger the scavenging process. Miller and others (2003) tested different manufacturing methods of probiotic yogurt as well as different packaging systems, and they evaluated their effect on the dissolved oxygen content during a normal shelf-life for yogurt (42 days). Best results were obtained by fermenting set-type yogurt in an oxygen-barrier container lined with an OS film. The initial dissolved oxygen concentration of 16 ppm was decreased to 3 ppm (normal container) and 1.7 ppm (OS-container) after the first day. It further decreased to 0.2 ppm and 0 ppm, respectively, after 42 days. The rapid oxygen reduction observed within the first day was highlighted by the authors to be of particular importance regarding the probiotic bacteria which require low oxygen concentration for post-fermentation, thereby leading to a product with increased health benefit. In practical

applications, it is important to note that OS-lidding films can only be successfully applied with high oxygen-barrier containers.

In ultra-high temperature (UHT) milk, oxygen scavengers have been shown to prevent the development of stale flavor. Perkins and others (2007) packaged indirectly processed UHT milk using packaging films containing a prototype $ZerO_2^{TM}$ OS film laminate. Thereby, the OS film significantly reduced the initial dissolved oxygen content of ~7 mg/L to ~3.8 mg/L during 14 weeks of room temperature storage (26 °C), compared to the control with ~5 mg/L. Significant reductions of 23-41% were also observed for stale flavor volatiles such as methyl ketones and aldehydes. Regarding lipid oxidation, a gradual increase in total free fatty acid levels was observed during the 14-week storage period of samples without OS (data of OS not published). However, the levels remained far below threshold values, indicating low lipolytic rancidity. As a consequence, the consumer panel failed to detect a significant difference in odor between the samples with and without OS. The authors concluded that sensory analysis would have better reflected the rancid off-flavor, however, a lack in regulatory approval for the OS prototype used in their study precluded taste-testing.

Unsaturated hydrocarbon dienes. Baiano and others (2004) incorporated an oxygen-scavenging copolyester-based polymer (Amosorb DFC 4020, ColorMatrix Europe Ltd, Knowsley, UK) into PET bottles. The oxygen-scavenging principle was based on a transition metal, such as cobalt salt, which catalyzed the reaction between oxygen and unsaturated hydrocarbon dienes that are linked to polyester (Cahill and Chen 2000). The authors evaluated the influence of the OS PET bottle on ascorbic acid degradation and browning in a

model system simulating citrus juice. With a 16-week storage period at 5 and 35 °C, the authors demonstrated that the use of OS bottles could significantly slow down the degradation kinetics of ascorbic acid and the browning reactions. Compared to glass jars and conventional PET bottles, the vitamin C loss in the OS bottles was half at 35 °C storage and even 3-4 times lower at the usual refrigerated storage of 5 °C. The authors reasoned the inferior results with PET and glass were due to the oxygen permeability of PET and the presence of pro-oxidant substances and catalysts in glass containers. The results demonstrated that glass containers could be advantageously replaced with polymeric bottles including an oxygen scavenger, particularly in the case of beverages containing ascorbic acid. However, the application of a real fruit juice instead of a model system would have been preferable. Using the above-mentioned OS polymer in the form of a cast-extruded monolayer-PET film, Galdi and Incarnato (2011) demonstrated the prevention of the browning of bananas. Fresh-cut banana slices were shown to have significantly less (~50%) color difference (ΔE) after three days wrapped in the OS film compared with the conventional PET film. Later optimizations of these OS PET films resulted in co-extruded multilayer films where the internal active layer was protected from fast oxidation by 2 external layers of pure PET (PET/OS-PET/PET) in order to increase the reaction time (Di Maio and others 2015).

Palladium. Many of the oxygen-scavenging systems that have been developed are still too slow for several food applications, such as boiled meat products, especially if they are packaged in slices. For such oxidation-sensitive products, removal of oxygen by conventional means is not generally achievable since light-induced discoloration in meat occurs within

hours, even at very low oxygen levels (0.5 until 0.1 vol.-%), depending on the product/headspace ratio (Andersen and Rasmussen 1992; Møller and others 2000; Nannerup and others 2004; Larsen and others 2006; Gibis and Rieblinger 2011; Böhner and others 2014; Hutter and others 2016); and most OS systems require several days to remove initial headspace oxygen (Matche and others 2011). Recently, a rapid OS system based on a catalytic system with palladium (CSP) has been developed. Palladium was coated on PET/SiOx-films using magnetron sputtering technology (Lohwasser and Wanner 2005; Yildirim and others 2010, 2015).This OS film was able to remove up to 2.5 vol.-% residual oxygen in food packages if hydrogen was included in the modified atmosphere (MA) of the packaging (Yildirim and others 2015). Due to its high efficiency, this film is particularly suitable for food products which are very susceptible to oxygen and where the oxidation reactions are very fast. Hutter and others (2016) showed that an implementation of this OS film in packaging prevented the discoloration of cooked cured ham. The OS film removed the 2 vol.-% initial oxygen in the headspace (160 cm^3) of MA-packed ham within 35 min after packaging. In this way, the color of the ham could be preserved and discoloration prevented for 21 days of storage, although packages were exposed to light 24 h/day. In contrast, samples packaged without OS film and stored in light significantly lost their redness within 2 hours of storage. The same OS film was applied to bakery products. For packaged breads, mold growth is the key factor limiting shelf-life. In this context, Rüegg and others (2016) applied the palladium-based OS film in MA packages of partially baked buns, toast bread slices, and gluten-free bread slices. At normal and modified atmosphere without OS film, visible mold growth was detected in all samples after 2 days with a simultaneous decrease in

491 headspace oxygen concentration. In contrast, in MA packages with OS film, mold growth was
492 retarded up to 8-10 days, resulting in a 3-4 fold longer shelf-life for all types of bread tested.
493
494 Apart from its high efficiency, the applied CSP also has some limitations. First of all,
495 hydrogen is required, therefore, the application of the CSP is limited to products packed
496 under modified atmosphere. As hydrogen concentrations >5.7 vol.-% in nitrogen are
497 considered as flammable, a maximum amount of oxygen to be removed was suggested to be
498 2.5 vol.-% (Yildirim and others 2015). Another drawback is that the catalytic activity of the
499 palladium-based system may be inhibited or even inactivated by volatile sulfur compounds
500 present in the headspace of certain packaged food products (Röcker and others 2017). With
501 regard to the safety of palladium, the EFSA published a scientific opinion on the safety
502 assessment of the palladium metal and hydrogen gas for use in active food contact
503 materials. The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing
504 Aids (CEF) concluded that *"the active substances palladium and hydrogen do not raise a*
505 *safety concern for the consumer when used as an oxygen scavenger in packages for foods*
506 *and beverages at room temperatures or below*. *Palladium should not be in direct contact*
507 *with food and should be incorporated in a passive structure impermeable to liquids which*
508 *prevents the migration at detectable levels"* (EFSA CEF Panel 2014).
509
510 Implementation of OS technologies into food packaging operations is often challenging as OS
511 systems are sensitive to environmental conditions, such as temperature and pH (Galdi and
512 others 2008; Solovyov 2010; Damaj and others 2014), or require humidity (Solovyov 2010) or
513 UV-light (Rooney 1999; Miller and others 2003; Zerdin and others 2003) to trigger the

oxidative reaction, or negatively interact with food volatiles (Röcker and others 2017). The incorporation of OS materials into polymer matrices may change the film properties, such as OTR or WVTR values (Matche and others 2011). This should be considered as a change in such properties may result in decreased quality of the food. Additionally, not only the active substances, but also all other materials used to incorporate active substances into the packaging should be safe. Finally, OS systems should not have any negative influence on the sensory properties of the food.

For the selection of a suitable OS system for a specific food application, factors influencing the oxidation kinetics, such as storage temperature, humidity, pH of the food and possible light exposure, should be considered. Information about initial headspace oxygen concentration, headspace volume, barrier properties of the packaging, as well as the minimum shelf-life, is also essential to determine the required OS capacity and OS rate. Finally, the cost of the OS packaging has to correspond with the benefit provided to the particular food product.

Moisture Scavengers

Moisture content and water activity are critical factors affecting the quality and safety of various types of foods (Labuza and Hyman 1998). For instance, many dry products are sensitive to humidity during storage, and even low relative humidity (RH) levels inside the packages may cause significant quality deterioration. Increase in moisture makes the products more prone to microbial spoilage and may cause alterations in texture and appearance, consequently reducing shelf-life (Labuza and Hyman 1998, Day 2008). For other products, such as fresh fish, meat, and fruit/vegetables, keeping a controlled high RH level

inside the package is beneficial in preventing drying. In addition, some excess liquid caused by drip loss is common for such fresh products like fish and meat. Consumers perceive liquid in a package as reducing the attractiveness of a product making it less desirable (Droval and others 2012).

Moisture control strategies in packaging can be divided into categories, such as moisture reduction (for example, by MAP through replacing the humid air in the headspace with dry modified atmosphere gas, or vacuum-packaging through the removal of humid air in the headspace), moisture prevention (by barrier packaging), and moisture elimination (by applying a desiccant/absorber). Only the latter category can be considered active, whereas moisture reduction and prevention are more passive strategies. Passive systems can include those that lower the humidity without any active ingredients, such as micro-perforated films (for example, Xtend® films, Israel) (Suppakul 2015), and other packaging materials that are inherently hygroscopic, such as those that are 100% cellulose-based (as defined in (EC) No 450/2009 (European Commission 2009)). Humidity levels inside packages can also be controlled through the appropriate selection of packaging materials with a high barrier against water vapor.

Active moisture scavengers can be further distinguished into 2 main types: RH controllers that scavenge humidity in the headspace, such as desiccants, and moisture removers that absorb liquids (Brody and others 2001). The latter can be applied in the form of pads, sheets, or blankets, and are typically placed underneath fresh products in different packaging concepts (MAP, vacuum, skin pack, and so on). They are applied for foods of high water

activity: fish, meat, poultry, fruits, and vegetables (especially cut products) (Vermeiren and others 1999, Day 2008). Thereby, drip loss increases with storage time and by increasing the exposed surface area, and longitudinal cutting of the muscle fibers (McMillin 2008). Such pads are mostly composed of porous materials, polymers (PP or PE), foamed and perforated PS sheets, or cellulose, combined with superabsorbent polymers/minerals/salts (polyacrylate salts, carboxymethyl cellulose, starch copolymers, silica/silicates) (Ščetar and others 2010). Moisture absorbing pads are not often considered to be active packaging. According to the EU Guidance to the Commission Regulation (EC) No 450/2009, "*Materials and articles functioning on the basis of the natural constituents only, such as pads composed of 100% cellulose, do not fall under the definition of active materials because they are not designed to deliberately incorporate components that would release or absorb substance*." However, moisture absorbing pads containing components that "*are intentionally designed to absorb moisture from the food*" and can be considered as active packaging (European Commission 2009). Absorbing pads can also be used in combination with antimicrobials (for example, Dri-Fresh®Fresh-Hold™ ABM, Sirane, USA), pH control agents and/or carbon dioxide generators/oxygen scavengers, to avoid certain shortcomings, such as odor generation or leakage.

Desiccants are used to control humidity in the packaging headspace. Examples of desiccants are: silica gel, clays, molecular sieves (synthetic crystalline version, such as from zeolite, sodium, potassium, calcium alumina silicate), humectant salts (such as sodium chloride, magnesium chloride, calcium sulfate), and other humectant compounds (such as sorbitol); as well as calcium oxide (Müller 2013, Day 2008). The absorption capacity of desiccants

584 depends on its water vapor sorption isotherm (Sängerlaub and others 2013b). Desiccants are
585 commonly placed into packages in the form of sachets, micro-porous bags, or are integrated
586 in pads. Some examples applied to food products are given in Table 3, however, pads are
587 excluded as they are already successfully applied on the market.
588
589 **Zeolites.** Zeolites have a significant tendency to attract moisture and can also release the
590 absorbed water without any change in the crystalline structure and moisture absorption
591 properties (Julkapli and Bagheri 2016). By virtue of these properties, natural nanozeolite can
592 be added to pulp/paper and plastic films to regulate the amount of moisture absorbed in
593 packaging (Julkapli and Bagheri 2016), however, its application is still limited to non-food
594 packaging (Wu and others 2010).
595
596 **Bentonite/sorbitol/calcium chloride.** One of the potential application of moisture
597 scavengers is packaging of mushrooms since the quality of the mushrooms is strongly
598 influence by the RH in the headspace. Humidity below 86% RH promotes moisture and
599 hence weight loss, mushroom senescence, and textural changes; on the contrary, 100% RH
600 promotes psychrophilic bacteria growth and also causes discoloration of mushrooms. The
601 optimal humidity in the headspace of packaging for mushrooms was found to be 96%
602 (Mahajan and others 2008). Mahajan and others (2008) investigated new packaging
603 concepts for fresh mushrooms (*Agaricus bisporus*) - a produce with a high moisture content
604 and short shelf-life (1-3 days at ambient temperature). In this study, combinations of fast-
605 absorbing ($CaCl_2$, KCl, and sorbitol, with moisture holding capacity 0.91 ±0.01 [g H_2O/g] in
606 120 h) and slow-absorbing moisture absorbers (bentonite/sorbitol, with moisture-holding

607 capacity 0.34 ±0.02 [g H_2O/g] in 120 h), were tested (Table 3). The best combination of
608 absorbers was found to be bentonite/sorbitol/$CaCl_2$ in proportions of 0.55:0.25:0.2 g/g
609 desiccant, respectively. The study revealed that the moisture holding capacity of the
610 scavenger is dependent on the relative humidity; it increased from 0.51 to 0.94 g water per g
611 desiccant when the relative humidity was increased from 76% to 96%. A change in
612 temperature (from 4 to 16 °C) did not have a significant influence on the moisture-holding
613 capacity. A positive impact of desiccant usage was observed, namely a decrease in the
614 moisture condensation inside the packaging, and improved transparency of the packaging
615 film. Mushrooms (250 g) packed with 5 g of bentotite/sorbitol/$CaCl_2$ desiccants resulted in a
616 lower browning index (BI 14.8) compared to those packed without desiccants (BI 18) after 5
617 days of storage at 10°C. However, for packages with higher levels of desiccants (10 or 15 g),
618 browning indices were greater due to the browning and excessive moisture loss. The
619 appearance of mushrooms packed with 5 g desiccants was also better since higher amounts
620 resulted in excessive moisture loss. After 5 days, the mushrooms packed with 5 g of
621 desiccants were still sellable on the market. The results show that the capacity of the
622 moisture absorbers had to be precisely tuned and product-adjusted to achieve an optimal
623 effect, as too high absorption capacities could result in a decrease in quality. Similar
624 experiments were performed by Azevedo and others (2011). A mixture of desiccants
625 consisting of calcium oxide/calcium chloride/sorbitol in a ratio of 0.5:0.26:0.24, respectively,
626 resulted in a moisture-holding capacity of 0.81 g water per g of desiccant. However, no food
627 application was evaluated in this study.
628

Poly(acrylic acid) sodium salt. Mbuge and others (2016) investigated the use of a food grade super-absorbent polymer (SAP) as desiccant for the drying of maize in order to reduce mold growth, and, consequently, aflatoxin contamination. The applied SAP, a cross-linked poly(acrylic acid) sodium salt powder, was placed into a porous “tea bag” membrane and integrated into sealed containers (material not stated) containing fresh maize with a water content of about 32%. After drying the maize to the optimal water content of 13 % at 40°C drying temperature, the lowest aflatoxin contamination was observed for the applications with SAP-to-maize-ratios of 1:5 and 1:1 resulting in aflatoxin contents of 33 or <3 ng/g, respectively. Aflatoxin contamination could be reduced to <4 ng/g, even at 20, 30 and 40 °C drying temperatures, with a 24 h frequency change of the SAP (1:5), complying with current Kenyan and European legislation that limit aflatoxin content to 10 and 4 ng/g, respectively. The application of poly(acrylic acid) sodium salt therefore shows potential for grain drying in reducing aflatoxin contamination, particularly in developing countries as it is a cheap and reusable solution.

Sodium chloride and hygroscopic ionomer. Langowski and others (2006) and Sängerlaub and others (2013b) developed salt-embedded, humidity-regulating trays consisting of a 3-layer structure: barrier layer, active layer with NaCl and sealing layer, to control the humidity in food packages. Thereby, the active layer was foamed and stretched to form cavities around the salt particles. Such humidity-regulating trays, made of a thermoformed multilayer structure containing a foamed hygroscopic ionomer Entira™ AS SD100 as an active layer, were used by Rux and others (2016) to pack tomatoes and strawberries (Table 3). Thereby, the active layer contained 0 or 12 wt% NaCl. When just water was packed, the

652 amount of water absorbed by the trays containing 0 and 12 wt% NaCl was 7.6 and 13.2 g,
653 respectively. In the presence of tomatoes or strawberries, the humidity produced by these
654 products was efficiently absorbed by the trays and no condensation effect was observed.
655 The trays containing 12 wt% NaCl best regulated the in-package RH below 97%. A slightly
656 higher product weight loss (2-3 wt% for strawberry, 1 wt% for tomatoes) compared to the
657 control PP trays (0.3-0.6 wt%) was observed. However, in this study, no other quality
658 parameters of the strawberries and tomatoes were evaluated. Similar weight loss has been
659 observed in the study of Rux and others (2015). They reported a water loss of 11.4 g for
660 packaged mushrooms (250 g) in similar humidity-regulating trays (PP/foamed and stretched
661 PP -NaCl/EVOH/PE) containing 18 wt% NaCl, compared to 6.7 g water loss for those packed
662 in standard PP trays, during a storage of 6 days at 7°C. In-package RH remained stable at 93%
663 during storage. After 6 days, mushrooms packed in humidity-regulating trays had a better
664 color appearance and gill exposure as well as less incidence of decay, compared to those in
665 the control PP trays. Singh and others (2010a, 2010b) also confirmed the water loss for
666 packed mushrooms with such humidity-regulating trays. An increase in the amount of NaCl
667 integrated in the trays from 6 to 18 wt% resulted in a weight loss within the range of 1.3 to
668 4.5 g for packaged mushrooms at 5 °C. Differences in moisture loss with the same type of
669 moisture-absorbing packaging may occur due to the different physiological state of the
670 product, storage temperatures, and packaging films used.
671
672 **Tamarind seed galactoxyloglucan.** Polysaccharides, such as galactoxyloglucan from tamarind
673 seeds, can be used as moisture-absorbing aerogels. Such aerogel-based packaging systems,
674 in combination with enzyme-based (galactose oxidase) oxygen-scavenger systems, were

shown to have capacities to absorb water and saline solution 40 times their weight (Gracanac 2015). With the absorption of drip, however, the absorption capacity was reduced to 20 times the initial weight. Nevertheless, galactoxyloglucan aerogels have been shown to have potential to be employed in moisture-absorbent materials for meat packaging applications.

Commercial moisture absorbers can be found in a variety of formats including as absorbing pads, such as Cryovac®Dri-Loc® (Sealed Air Corporation, USA), Thermasorb (Thermasorb PVT Ltd., Australia), and MeatGuard® or MeatPad® (McAirlaid's Inc., USA); absorbing films, such as Pichitto/Pichit (MTC Kitchen, Japan), MoistCatch™ (Kyodo Printing Co., Ltd., Japan), and Active Film™ (CSP Technologies, USA); absorbing paper, such as Onyx Desiccant Paper (Onyx Specialty Papers, Inc., USA); absorbing pouches, such as Humidor Bag (Boveda Inc., USA), and trays, such as Fresh-R-Pax® (Maxwell Chase Technologies, LCC, USA).

As described in this section, various moisture scavenging systems can be applied to preserve quality and prolong the shelf life of food. The examples presented above underline the importance of product-adjusted moisture scavenging systems for food applications. The most well-established and commercially well-recognized technologies incorporate the use of sachets, pouches (including desiccants) or pads – devices that do not interfere with the structure of external packaging materials. Implementation of moisture absorbers/controllers in other forms, such as in the structure of packaging or as a coating, for commercial food product applications (fresh fruits, vegetables, fish and meat) is still under development and future research is expected to be focused in this area (Restuccia and others 2010).

Ethylene Absorbers

Ethylene (C_2H_4) is a growth-stimulating hormone (plant growth regulator) accelerating ripening and senescence through increasing the respiration rate of fresh and minimally processed climacteric produce and shortening the shelf-life during postharvest storage. Ethylene also accelerates chlorophyll degradation rates, especially in leafy products, and enhances excessive softening of fruits (Saltveit 1999; Ozdemir and Floros 2004). For these reasons, the removal of ethylene from the product environment by application of ethylene scavengers slows ripening and senescence, thereby enhancing quality and prolonging shelf-life.

Potassium permanganate. Ethylene scavenger systems involve either inclusion of a small sachet containing an appropriate scavenger in the packaging or incorporation of an ethylene absorber in the film structure. The sachet material should be highly permeable to ethylene, allowing diffusion through it. The most commonly used active component of the sachet is potassium permanganate ($KMnO_4$) in order to oxidize/inactivate ethylene (Floros and others 1997; Ayhan 2011; Llorens and others 2012) However, $KMnO_4$ is never used in direct food contact due to its high toxicity (Martínez-Romero and others 2007).

Minerals. Another ethylene-scavenging system is based on the use of finely dispersed minerals, such as zeolite, active carbon, or pumice. These minerals could be incorporated into a plastic film structure commonly used in fresh produce packaging (De Kruijf and others 2002). Such minerals are intended to scavenge ethylene and also modify the gas

721 permeability of the film so that carbon dioxide can diffuse faster and oxygen can enter more
722 readily than through pure polyethylene to obtain an equilibrium atmosphere (De Kruijf and
723 others 2002; Esturk and others 2014).
724
725 **Metals and metal oxides** are also good candidates for ethylene removal. Photoactive TiO_2 is
726 reported to oxidize ethylene into water and carbon dioxide. Since metal oxides are activated
727 by either UV light, visible light or both, the negative effect of UV exposure on food quality
728 should be considered. Nano-silver is also claimed as an ethylene blocker; however, it has
729 been tested in absorbent pads which were placed in trays of fresh-cut melon and not in the
730 packaging structure (Hu and Fu 2003; Fernández and others 2010). Palladium-based
731 scavengers are shown to have good ethylene adsorption capacity, but they are mostly tested
732 as sachets in packages (Abe and Watada 1991; Bailén and others 2006, 2007; Cao and others
733 2015) or in a storage room (Martínez-Romero and others 2009), not in the structure of
734 packaging films. The high cost of palladium has been assumed to limit its industrial
735 application (Martínez-Romero and others 2007). Abe and Watada (1991) reported that
736 charcoal with palladium chloride as an absorbent, present in paper sachets and not in the
737 packaging structure, was effective in preventing ethylene accumulation, reducing the
738 softening in fresh-cut kiwifruit and bananas, and chlorophyll loss in spinach leaves, but not
739 effective in broccoli pieces. It was also effective in absorbing most of the ethylene during 3
740 days of storage for kiwifruit slices and banana sections at 20 °C. An ethylene concentration
741 of 0.4 ppm in the trays of broccoli and spinach was effectively absorbed by the ethylene
742 absorbent. In this study, 10 g paper packets containing ethylene absorbent were placed in

743 metal trays with a glass cover, however, it was acknowledged that this type of high barrier
744 packaging is not suitable for products that respire.
745
746 Sothornvit and Sampoompuang (2012) incorporated activated carbon (30%) with 0.3% of the
747 polysaccharide glucomannan into paper made of rice straw. The active material adsorbed
748 0.69 μL/L ethylene with a scavenging capacity of 77%. The ethylene adsorption capacity per
749 surface area was calculated as 34.2 μL/L/m. Therefore, it was suggested that a separate bag
750 or wrapper or a laminate inside a carton might have the potential to extend the shelf life of
751 ethylene-sensitive products, such as banana, mango, tomato, and apple. However, no food
752 application was evaluated in this study. There has also been a further study on cardboard
753 coated with polylactic acid and ethylene scavengers (clinoptilolite, sepiolite, sepiolite
754 permanganate) designed as an active packaging for fresh fruits and vegetables. However, in
755 this study, there was no application to prove the effect in a real food system (Taboada-
756 Rodríguez and others 2013).
757
758 The incorporation of scavengers in packaging films may be a better option to solve sachet-
759 related problems. Ethylene scavengers could either be embedded into a solid, dispersed in
760 plastic, or incorporated into various layers of the packaging (Ozdemir and Floros 2004).
761 However, there has been only limited research into the application of ethylene absorbers in
762 the structure of packaging films. The main focus of the following section is the application of
763 ethylene scavengers incorporated into the actual packaging material, rather than in sachet
764 format, for fresh produce. Ethylene scavenger can prolong the shelf life of climacteric fruits,
765 such as apples, kiwifruit, apricot, bananas, mango, cucumber, tomato, and avocados, and

766 vegetables, such as carrots, potatoes, and asparagus (De Kruijf and others 2002). The list of
767 different produce packaged with different packaging film and ethylene absorber and the
768 benefits of such systems are presented in Table 4.
769
770 **Nano-particles.** Nano-TiO_2 is reported to oxidize ethylene into H_2O and CO_2 (Han and Nie
771 2004). Yang and others (2010) tested PE blended with nano powders of Ag, TiO_2, and kaolin
772 for preservation of fresh strawberries at 4 °C for 12 days. Results showed that active PE with
773 nano-powders maintained physicochemical and physiological quality and sensory attributes
774 of strawberry better than the control (PE). Active packaging decreased the rate of fruit decay
775 (to 16.7% for nano-packaging and 26.8% for normal packaging), maintained the content of
776 total soluble solids, preserved ascorbic acid, and reduced the malondialdehyde content (to
777 66.3 μmol/g for nano-packaging and 75.4 μmol/g for normal packaging), and enzyme activity
778 of polyphenol oxidase and pyrogallol peroxidase. However, the gas composition including
779 ethylene in the headspace was not monitored in this study and there is no indication of shelf
780 life.
781
782 Chinese bayberries were packaged by Wang and others (2010) with active PE including 30%
783 nano powder of Ag, TiO_2, and kaolin-clay or treated with hot air or the combination of hot air
784 treatment and nano-packaging, and stored at 1 °C and 80-90% RH for 8 days. Results showed
785 that the application of hot air (48 °C for 3 h) and/or active packaging reduced the incidence
786 of green mold decay (from 75.5% to 34.6% for active packaging and to 22.7% for hot air
787 treatment and to 14.8% for the combined treatment), fruit respiration, and ethylene
788 production, and maintained fruit firmness compared to the control (fruit directly packed in

PE with no heat treatment) for 8 days of storage. The respiration rate and ethylene production of the combined treatment of hot air and active packaging were 49.6% and 25.9%, respectively, which were lower than the control. This study suggested that the combined treatment was more effective in maintaining the quality of Chinese bayberries than heat treatment or nano-packaging alone.

Li and others (2009) studied the effect of active packaging produced by blending nano powders of nano-Ag, nano-TiO_2, and kaolin with polyethylene for the preservation of Chinese jujube. The active packaging improved the physicochemical and sensory quality of the product compared to polyethylene without nano-powder (control). Application of active packaging significantly reduced fruit softening, weight loss, browning, and climactic evolution during 12 days of storage. An important index of rate of browning of the product was reduced from 0.7 to a lower level of 0.6 on day 12. The ethylene production rate increased initially and then declined for all treatments. The maximum ethylene content was reported as 17.6 μL/kg h for the control on the third day and 9.2 μL/ kg h for nano-packaging on the sixth day of storage. The active packaging is recommended for Chinese jujube to improve quality, however, a specific shelf life was not indicated.

Hu and others (2011) studied the effect of PE blended with nano-Ag, nano-TiO_2, and montmorillonite on the quality of ethylene-treated mature kiwifruit at 4 °C for 42 days. Weight loss, softening, color variation, and Brix degrees (°Brix) of kiwifruit were significantly reduced by 22.7%, 124.8%, 23.5% and 14.4%, respectively. Ethylene concentrations in the headspace were 39.5 μL/L and 16.8 μL/L for the control and nano-packaging, respectively,

on storage day 42. For kiwifruit, 30 μL/L was reported to cause unacceptable softening. The researchers stated that nanocomposite packaging was effective in inhibiting ethylene production (57.4% of lower headspace ethylene in active packaging), preventing physiological changes, and delaying ripening, however, no specific shelf life was indicated. A lower level of ethylene production was related to the synergistic effect of nanoparticles which decompose or oxidize ethylene into water and carbon dioxide (Li and others 2009). Li and others (2011) tested poly(vinyl chloride) (PVC) film coated with nano-ZnO powder on fresh-cut 'Fuji' apple at 4 °C for 12 days. Nano-coated PVC film reduced fruit decay rate and enzyme activity, retarded ethylene production, maintained °Brix and titratable acidity compared to uncoated PVC (control). Maximum ethylene content was reported as 40 μL/ kg day for nano-packaging on the ninth day and, 70 μL/kg day for the control on the sixth day of storage. The browning index was significantly reduced from 31.7 to 23.9 on day 12, maintaining the initial appearance. The activity of polyphenol oxidase was 9.6 U/g min in active packaging and 21.5 U/g min in normal packaging on day 9. The authors reported that the nano-packages had more oxygen and less carbon dioxide in the headspace compared to the control, indicating the lower respiration in the nano-coated PVC. Nano ZnO is reported to have similar physical properties to TiO_2 which oxidizes ethylene into water and carbon dioxide under UV irradiation (Han and Nie 2004).

Zeolite-based minerals. Zeolite-based ethylene absorbers are good candidates for commercial use. The most characteristic property of zeolites is their porous three-dimensional structure with cation exchange, adsorption, and molecular sieving properties. Therefore, zeolites have been used in many industrial and agricultural applications, including

as an ethylene-absorbing additive incorporated into packaging films. There are various reports that incorporation of zeolites increases gas permeability of packaging films by means of their crystalline porous-three-dimensional framework structure (Süer and others 1994; Kittur and others 2005; Zhao and others 2011). Esturk and others (2014) successfully applied low-density polyethylene (LDPE) bags with ethylene absorber (8% Tazetut® master batch, an inorganic product containing 50% of various alumino-silicate minerals (zeolite)) to broccoli florets under passive modified atmosphere and stored at 4 °C for 20 days. The authors stated that spoilage occurred quickly in unpackaged broccoli (control) illustrated by chlorophyll degradation, stem-hardening, and mass loss of 41.5% on day 20, which was less than 1% for packaged applications. The product (control) was unacceptable for the sensory panel after 5 days. However, the quality loss was significantly reduced in active LDPE bags with an ethylene absorber. Ethylene concentration was 61.8 ppm in the control LDPE and 0.33 ppm in active LDPE at the end of the storage. Thus, packaging with zeolite-based active films extended the shelf life of broccoli up to 20 days, compared to a 5-day shelf-life for the unpackaged product.

The quality of kiwifruit packaged with HDPE bags including a sachet with $KMnO_4$ impregnated zeolites at 4 °C for 31 days was reported by Küçük (2006). 0.2 mL $KMnO_4$/g zeolite was impregnated with zeolites of 1-3 mm in size. $KMnO_4$ impregnated zeolites were added into the HDPE bags as 5 and 10% of the amount of kiwifruit. Fruits were firmer and had a higher vitamin C content in zeolite-containing HDPE films compared to the control (60.67 mg/100 mL of vitamin C for fruits packaged using HDPE with 5% zeolite and 47.37 mg/100 mL for the control on day 31). There was no significant difference reported for color

values L*, a*, and b* (L* indicating lightness, a* chromacity on a green (-) to red (+) axis, and b*chromacity on a blue (-) to yellow (+) axis) between HDPE bags with and without zeolite. The ethylene measurements for each treatment and the shelf life were not published in this publication.

Boonruang and others (2012) tested 4 different packaging films for mango at 12 °C. The tested materials were non-perforated, highly gas-permeable film, non-perforated highly gas-permeable film with ethylene-absorbing property, micro-perforated highly gas-permeable film and common low-density polyethylene film. The non-perforated film with ethylene absorber extended the shelf life of mango to 40 days at 12 °C, compared to 35 days with the non-perforated, highly gas-permeable film, 30 days with the microperforated film , and 5 days with the common low-density polyethylene. The film with ethylene absorber reduced weight loss, maintained firmness, and there was no sign of decay during storage. Low ethylene concentrations (<3.5 µL/L) were reported in mangoes with the different packaging films. Ethylene production in the microperforated packages was higher than that in the non-perforated and non-perforated with ethylene absorber packaging. The ethylene-absorbing characteristics of the material delayed the ripening process of mangoes. A further study shows that zeolite-added LDPE bags were applied to ripe kiwifruits successfully establishing an equilibrium atmosphere in the headspace in 5 days, however, the control material with no ethylene absorber did not reach equilibrium (steady state oxygen and carbon dioxide) during 20 days of cold storage at 4 °C. A minimum shelf life of 20 days was suggested for kiwifruits using zeolite-incorporated LDPE bags. Ethylene measurement is not reported in this study (Ayhan 2016).

Jacobsson and others (2004) tested 4 different materials on fresh broccoli at 2 storage temperatures (4 and 10 °C). Among the materials tested, one material was LDPE-based with pouches containing a commercial sachet (Ryan Instruments, The Netherlands) to absorb ethylene, and the other material was commercial LDPE film impregnated with a natural hydroscopic mineral produced by PEAKfresh® (USA). Results showed that LDPE pouches with a sachet provided 11 days and LDPE incorporated with ethylene absorber provided 12 days of shelf-life at 4 °C. But at 10 °C, the commercial LDPE bags incorporated with ethylene absorber provided the longest shelf-life of 9 days compared to 6 days for sachet application for broccoli. LDPE film impregnated with a natural hydroscopic mineral resulted in a lower loss in weight, better color and texture, while the chlorophyll content was maintained. This publication does not report any ethylene measurements.

Commercially-available ethylene-scavenging films in the market are mostly zeolite-based such as Evert-Fresh® (Evert-Fresh Corporation, USA), PEAKfresh® (PEAKfresh®, USA), Profresh® (E-I-A Warenhandels GmbH, Austria) and Bio-Fresh™ (Grofit Plastics, Israel). The main limitation of these films is opacity and, thus, these plastics are mostly colored (Martínez-Romero and others 2007; Ayhan 2013). PEAKfresh® is a polyethylene bag impregnated with minerals to absorb ethylene and moisture. Evert-Fresh® absorbs ethylene, ammonia and carbon dioxide. An additive made with low-density polyethylene (LDPE) to absorb ethylene, ethanol, ethyl acetate, ammonia, and hydrogen sulfide is incorporated in Profresh®. Bio-Fresh™ is a film used in combination with modified atmosphere packaging to absorb various substances arising from the ripening process. Active and intelligent systems

are governed in Europe by regulations (EC) No 1935/2004 and 450/2009 (European Commission 2004, 2009). The use of permanganate as active agent in contact with food is not permitted in Europe (Pereira de Abreu and others, 2012).

Packaging materials integrated with ethylene-removers in the packaging structure are still limited in commercial applications. The use of these materials for a broad spectrum of fresh products is also limited compared with other active packaging applications reported in the literature. The main principle for the successful packaging of fresh and fresh-cut produce is that the gas permeability (oxygen and carbon dioxide) of the packaging film and respiration rate of the produce should correspond allowing gas equilibrium in the headspace, as well as removal of ethylene from the package environment (Ayhan 2013). The application of adequate oxygen and low carbon dioxide-modified atmosphere packaging combined with ethylene absorber could provide further benefits to control the product metabolism and increase the shelf life of fruits and vegetables compared to the application of solely MAP. However, it should be noted that packaging parameters should be designed to be produce-specific, since each produce varies in respiration rate, ethylene production rate, and ethylene sensitivity, and hence the requirements for packaging and storage vary.

Antioxidant Releasers

There has been increased activity in the development of antioxidant-releasing packaging for food applications during recent years. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely used in food packaging to prevent lipid oxidation. There is now growing interest in the inclusion of natural

antioxidants such as polyphenols, tocopherols, plant extracts, and essential oils to active packaging materials (Nerín and others 2006; Park and others 2012; Barbosa-Pereira and others 2014; Marcos and others 2014). Some recent developments in this field have been summarized in Table 5.

Torres-Arreola and others (2007) reported a delay in lipid oxidation and protein denaturation in fresh sierra fish fillets through the incorporation of BHT into LDPE packaging. Compared to LDPE films, samples packed in BHT-LDPE films demonstrated lower lipid oxidation, expressed as thiobarbituric acid-reactive substances (TBARS) values (4.20 ± 0.52 vs 11.95 ±1.06 mg malonaldehyde (MDA)/kg), peroxide index values (7.20 ±1.38 vs 15.15 ±1.48 meq/kg), and free fatty acid contents (7.98 ± 0.43 vs 11.83 ± 1.26 of oleic acid). Fillets packed in BHT-LDPE packaging films also inhibited less tissue damage and better retained firmness than fillets packed in LDPE. However, the presence of synthetic antioxidants in food is being questioned, therefore, the alternative approach that is being widely studied is the use of natural antioxidants such as α-tocopherol. Poly(lactide-co-glycolide) films loaded with 2% α-tocopherol, or a combination of 1% BHT and 1% BHA, were used to evaluate the stability of dry whole milk and dry buttermilk (Van Aardt and others 2007). BHT and BHA have a higher volatility than α-tocopherol, therefore it was expected that they would be more suitable for dry food applications. However, in this study, α-tocopherol offered the same antioxidant protection for whole milk powder exposed to light and oxygen. In a further study, sealable multilayer films (HDPE/EVOH/LDPE) manufactured with an inner LDPE layer containing 4% of α-tocopherol delayed the lipid oxidation of whole milk powder at 30 and 40 °C (Granda-Restrepo and others 2009b). Similarly, sealable LDPE films containing 1.9 and 3%

of α-tocopherol maintained the oxidation stability (hexanal content) of corn oil for 16 weeks at 30 °C, compared to 12 weeks for the oil in a control bag without antioxidant (Graciano-Verdugo and others 2010). Manzanarez-López and others (2011) reported that poly (lactic acid) films containing 2.58% of α-tocopherol were also able to delay the induction of the oxidation, measured as peroxide value, of soybean oil at 20°C (max. values of 9.9 vs 19.5 meq/kg), 30°C (max. values <10 vs 27.5 meq/kg), and 40°C (max. values of 13.5 vs 33.9 meq/kg). Meanwhile, Torrieri and others (2011) observed that with the combined use of MAP and LDPE-embedded α-tocopherol packaging, lipid and fat oxidation in fresh bluefin tuna fillets could be reduced. Several natural antioxidant products (TOCOBIOL®-PV, NUTRABIOL®-T90, NUTRABIOL®-T50 PV) containing tocopherols incorporated into LDPE films inhibited lipid oxidation of salmon muscle up to 40% during storage, hence being suitable for use in extending the shelf life of salmon (Barbosa-Pereira and others 2013).

Active antioxidant food packaging films produced by incorporating 4.6% of the natural flavonoid quercetin into EVOH matrix showed enhanced lipid oxidative stability, as demonstrated by a lower peroxide index (12 vs 27 meq/kg) and a reduction in TBARS values by 25% during storage time (López-de-Dicastillo and others 2012b). Catechin, an active component of green tea with properties similar to those of quercetin, was shown to be an effective antioxidant ingredient to retard the oxidation of sunflower oil and fried peanuts (López-de-Dicastillo and others 2012a). Fried peanuts stored in sealed bags manufactured with active films containing 0.33 and 1.34% of catechin at 37 ºC for 40 days resulted in a strong reduction in hexanal content released into the headspace up to 25 days, after which the hexanal increased at the same rate as in the control sample. Additionally, the peroxide

index was used to monitor the effect of the films on the oxidation of sunflower oil over 5 months. On exposing sunflower oil to the films, the peroxide values demonstrated that the films actively protected the oil. Moreover, the films with quercetin (0.76 and 4.01%) were more effective compared to those with catechin due to the higher solubility of quercetin in this product, as well as its higher antioxidant capacity. The reported results using accelerated shelf life testing (37 °C) to estimate the antioxidant potential of the films. However, the antioxidant performance under real storage conditions needs to be evaluated before commercial implementation. Another issue to be addressed would be the inclusion of EVOH in a multilayer system in order to protect the system from water and to reduce costs, while maintaining antioxidant activity. Other nonvolatile antioxidants such as ascorbic, ferulic, and citric acids incorporated into polymers such as sealable EVOH and sealable cornstarch/linear LDPE films demonstrated antioxidant activity when in contact with brined sardines and ground beef (López-de-Dicastillo and others 2012b; Júnior and others 2015).

Antioxidant packaging systems containing volatile extracts, essential oils, or active components of plants or spices have been developed to improve quality and to extend the shelf life of various food products. Thymol, carvacrol, and eugenol incorporated into sealable corn-zein-laminated linear LLDPE films were used for fresh ground beef packaging and effectively inhibited lipid oxidation and had a positive effect on the stability of beef patties during storage (Park and others 2012). The active linear LDPE composite film containing 1 wt% of the phenolic antioxidant resveratrol, which is naturally produced by plants under stress conditions, showed strong antioxidant activity, reduced lipid oxidation by 34.7%, and extended the shelf life of fresh meat stored at 4 °C by a few days (Busolo and Lagaron 2015).

Antioxidant films obtained from biomaterials containing green tea extract have been demonstrated to improve oxidative stability of pork meat products (Siripatrawan and Noipha 2012; Yang and others 2016). Yang and others (2016) reported a stronger antioxidant capacity of films obtained with 0.5% of green tea extract compared to those obtained with oolong and black tea extracts. Specifically, at the end of 10 days storage of pork meat, the TBARS value of the control sample was 1.64 mg MDA/kg, whereas the TBARS values of the samples wrapped with film containing green tea extract, oolong tea extract, and black tea extract were 0.93, 1.16, and 1.27 mg MDA/kg sample, respectively. In another study, Lorenzo and others (2014) reported that multilayer barrier films containing oregano essential oil (2%) were more effective in preventing lipid oxidation of foal meat packed in MAP (80:20, O_2:CO_2) than those with green tea extract (1%). An interesting investigation by Carrizo and others (2016) reported radical-scavenging capacity of sealable multilayer films, in which green tea extract was added to the laminating adhesive and thus not in direct contact with the packaged food (peanuts and cereals covered with chocolate). According to the authors, this packaging system was able to protect food against oxidation during a long-term period of 16 months. It is important to highlight the industrial relevance of this study, since the use of commercial packaging materials would facilitate industrial implementation of this technology.

Other authors have explored the application of packaging materials containing rosemary and oregano extracts in direct contact with muscle foods as active packaging systems. Antioxidant films containing rosemary and oregano extracts showed improved oxidative

stability of lamb and beef meat (Nerín and others 2006; Camo and others 2008, 2011). Oregano extracts included in the films were more efficient in preventing oxidation of lamb meat than rosemary extracts, they extended fresh odor and color from 8 to 13 days compared to the control (Camo and others 2008). Some authors have studied the impact of antioxidant packaging in preventing high-pressure-induced lipid oxidation. Bolumar and others (2011, 2016) developed LDPE films coated with rosemary extract that were able to protect meat patties from high-pressure-processing-induced lipid oxidation and consequently extend the shelf life. More specifically, the lipid oxidation of chicken breast patties submitted to high-pressure treatment and stored at 5 °C was higher in the surface part of samples and the active packaging delayed oxidation it up to 25 days demonstrated by lower peroxide values (7.2±1.38 vs 15.15±1.48 meq/kg), FFA (7.98±0.43 vs 11.83±1.26% oleic acid), and TBARS (4.20±0.52 vs 11.95±1.06 mg MDA/kg) (Bolumar and others 2011).

The use of by-products from the food industry as a source of antioxidants for food packaging has also been explored as a means of providing added value to these residues. Packaging of beef with LDPE film coated with a brewery residual waste extract was able to reduce lipid oxidation by up to 80% during cold storage (Barbosa-Pereira and others 2014). Barley husk, another waste product obtained from the brewery industry, also proved to be effective in slowing down lipid hydrolysis and improving the oxidative stability in blue shark muscle (Pereira de Abreu and others 2011). Meanwhile, anthocyanins from wine grape pomace, beet root residue powder, and mango and acerola pulp incorporated into sealable biodegradable films had a protective effect on sunflower and palm oil oxidation (Souza and others 2011; Oliveira and others 2016; Stoll and others 2016). For example, a sunflower oil

control sample directly exposed to the air and light reached a peroxide index of 65.8 meq/kg after 3 days, while the samples stored in cassava starch film bags prepared with encapsulated anthocyanins presented lower values (4.7-28.7 meq/kg) (Stoll and others 2016). Similarly, a lower peroxide index, which was significantly different from that of the control (oil with no packaging), was detected in palm oil packed in cassava starch films with high concentrations of mango and acerola pulp additives (Souza and others 2011). However, it was found that vitamin C in acerola pulp acted as a pro-oxidant agent, which suggests that the use of components rich in vitamin C should be avoided.

Extensive research on the use of antioxidant packaging systems to prevent food oxidation has been conducted. However, most of the reported studies fail to validate the efficiency of the antioxidant packaging systems in real commercial food applications and do not consider their target market and consequent legal status. Therefore, to favor the industrial implementation of this technology, it is essential to study real food packaging systems. Research efforts should focus on the use of packaging materials obtained through scalable film processing techniques (such as extrusion or coating vs solvent-casting), packaging materials with suitable barrier properties and formats for the studied food product, industrial packaging techniques (such as MAP or vacuum vs wrapping), effect on sensory properties of food, and validation using real storage conditions.

Carbon Dioxide Emitters

The antimicrobial effect of CO_2 is thoroughly documented in the literature (Kolbe 1882; Valley 1928; Haas and others 1989; Debs-Louka and others 1999). CO_2 is soluble in the

aqueous and fatty phases of food products and the antimicrobial effect is highly dependent on the rate of solubility and amount of CO_2 dissolved in the food product. The solubility of carbon dioxide increases with decreasing temperature (Devlieghere and others 1998; Devlieghere and Debevere 2000) and also varies for different food products depending on the properties of the food such as surface area, pH and composition (water, fat, protein) (Chaix and others 2015). The antimicrobial effect has been found to be proportional to the partial pressure of the gas (Blickstad and others 1981). In terms of food packaging, this implies that the total amount of CO_2 present in the headspace of the package is crucial for the effect. The concept of a CO_2-releasing device to be implemented in modified atmosphere packages (MAP) to maintain high headspace levels of CO_2 during storage, and thereby, facilitate smaller package volumes (lower gas to product (g/p) volume ratio), and prolonged shelf life was introduced in the 1990s.

The implementation of CO_2 emitters in MA-packages may allow for increased filling degree, reduced package sizes, improved transport efficiency, and a net reduction in environmental impact. The release of carbon dioxide from a tuned emitter system may also prevent packaging deformation as it compensates for CO_2 absorption into the food product in the initial stages of storage. In this way it counteracts formation of negative pressure in MA-packages that increase the drip loss of the product, which may give the packages an unattractive appearance to the consumer (Holck and others 2014). Further, inhibition of growth of spoilage bacteria and prolonged shelf life for fresh food products at sustained high CO_2 levels in the packages will have a knock-on effect in the form of a reduction in food waste, an issue gaining increasing attention and priority in western parts of the world.

The emitters usually come in the form of a pad or sachet, in many cases as a combined liquid absorber. The active ingredients inside the absorbent pad react when the pad absorbs liquid that is seeping out of the product, resulting in the release of CO_2. Over the last decade, the field of CO_2 emitters has advanced significantly, reflected in increased research activity and sale of commercial CO_2 emitters. Table 6 lists the CO_2 releaser technologies available for food preservation to date, as well as their applications and benefits to specific food products. In the literature, there are several reports of the use of ferrous carbonate in carbon dioxide emitters (Rooney 1995; Sivertsvik 2003; Restuccia and others 2010). However, documentation and descriptions of the technology principle, benefits, and food applications are scarce. Other concepts have also emerged, such as the technology applied in the emitter Verifrais™ (SARL Codimer, Paris, France). The active ingredients in the Verifrais® system are sodium bicarbonate and ascorbic acid (Rooney 1995; Kerry 2014). There are also examples of (commercial) combined O_2 scavengers and CO_2 emitters on the market, such as Ageless® G (Mitsubishi Gas Chemical Co., Japan) and FreshPax® M (Multisorb Technologies Inc, USA). These systems are based on either ferrous carbonate or a mixture of ascorbic acid and sodium bicarbonate (Coma 2008).

One of today's most well documented CO_2-releasing systems is based on a combination of the active substances sodium bicarbonate and citric acid. Several scientific publications document the effect of this CO_2-releasing system. Amongst the first reports in the literature of such applications is a study from 1995 (Bjerkeng and others 1995). In this study the effect of CO_2-emitter (non-commercial) on, for example, the microbial and sensory shelf life of cod

fillets in MA-packages (70% CO_2, 30% N_2) and vacuum was investigated. The CO_2 level in the MA-packages (g/p ratio not given) with emitter decreased to approximately 40% after a few days of storage and thereafter increased and fluctuated around 50% CO_2 for the remaining storage time. For the vacuum-packages with emitter, the CO_2 level increased to 50% after one day of storage and reached 80% at day 15 (very limited headspace volume available). For the control (MAP and liquid absorber) the CO_2 level rapidly dropped to 20% after one day of storage. The cod fillets packaged in modified atmosphere and vacuum with emitter were found to have a sensory shelf life, based on ammonia-like odor, of 11 days, compared to 7 days for the control (MA-packaged with liquid absorber), supported by measured trimethylamine (TMA) levels and microbial analyses (total viable counts (TVC) and H_2S-producing bacteria).

In a study by Hansen and others (2007), the effect of the same emitter system was investigated, also in the packaging of cod fillets, looking at the simultaneous effect of high CO_2 and O_2 level and different g/p ratio. Packaging in modified atmosphere (60% CO_2, 40% O_2) with CO_2 emitter at a low g/p ratio (1.3/1.0) resulted in extension of shelf life (14 – 21 days total) both in terms of sensory properties (odor and appearance assessment) and bacterial growth when compared to vacuum-packaging (7 – 14 days total shelf life). The shelf-life obtained with the emitter was, however, comparable to that of cod packaged in MAP at a high g/p ratio (3.9/1.0) without emitter. For the MA-packages (both g/p 1.3/1.0 with emitter and g/p 3.9/1.0 without emitter), the dominating bacteria at the end of the storage time were different species of *Carnobacterium* and some *Photobacterium*. In the MA-packages with a g/p ratio of 1.3/1.0 including an emitter, the level of headspace CO_2

1134 increased to about 70 – 80% during the storage period, effectively compensating for the CO_2
1135 absorbed by the product, whilst for MA-packages with a g/p of 3.9/1.0 without emitter, the
1136 CO_2 level dropped to 35-40% during the storage time. In a recently published study (Hansen
1137 and others 2016), cod fillets in vacuum-packages with a CO_2 emitter displayed a shelf-life
1138 extension of 2 days (9 days total) compared to vacuum-packages with a regular liquid
1139 absorber. However, the longest shelf life (13 days) was obtained for the combination MAP
1140 (60% CO_2 and 40% N_2) and CO_2 emitter, based on sensory (odor and appearance assessment)
1141 and microbiological evaluation (TVC, H_2S-producing bacteria counts, lactic acid bacteria
1142 counts (LAB), and microbiota analysis). With a CO_2 emitter present in the MA-packages, the
1143 headspace level of CO_2 (g/p 1.6/1) was kept stable at about 35 – 37% once the CO_2
1144 absorption into the product had reached equilibrium (after 1 day of storage). For the MA-
1145 packages (g/p 1.6/1) without emitter, the CO_2 level dropped to 26 – 27% after equilibrium.
1146 Distinct differences in TVC were measured for the different packaging methods; after 15
1147 days of storage the cod fillets in vacuum had a TCV of log 7.1 cfu/g, while the number for the
1148 cod in MA-packages and MAP with emitter was log 6.4 cfu/g and log 5.5 cfu/g, respectively.
1149 In the studies described above, the laboratory-type emitters were custom-made; the ratio
1150 between citric acid and sodium bicarbonate was adjusted to the pH of the food product.
1151
1152 The impact of the combined CO_2 emitter and liquid absorber (laboratory-type, custom-
1153 made) on the quality and shelf life of fresh salmon has been thoroughly documented
1154 (Hansen and others 2009a, b, c). Hansen and others (2009a) demonstrated the effectiveness
1155 of a laboratory-type emitter in reducing the g/p ratio for MA-packages (60% CO_2, 40% N_2) of
1156 salmon fillets without compromising the shelf life (based on microbial, sensory, and textural

analysis). For the MA-packages (g/p 3/1) without emitter, the CO_2 level dropped to 40% 4 days after packaging and then stabilized. For the MA-packages (g/p 1/1) with emitter, the CO_2 level displayed an initial drop to about 45% (day 1), but subsequently the level increased and reached 65 – 70% during the storage time. The measured TVC levels for the salmon packaged in MAP with and without emitter were comparable and the obtained shelf life for the two packaging methods was the same. The TVC of the MA-packaged samples (with and without emitter) reached a level of log 5 – 6 cfu/g after 15 days of storage, while for the vacuum-packaged salmon, the same bacterial counts were measured 7 – 10 days into storage. The results illustrate that a CO_2 emitter can allow for a more sustainable packaging of fresh fish products with a significant reduction in package sizes and hence amount of packaging material, since a comparable shelf life can be obtained at significantly reduced g/p ratio.

The emitter system (laboratory-type) has also been studied for different meats. In a study by Pettersen and others (2014), the effect of different packaging methods was evaluated for fresh reindeer meat. The study documented prolonged sensory shelf life (odor evaluation) for meat packaged in modified atmosphere (60% CO_2, 40% N_2) with and without CO_2 emitter of 21 days, compared to 17 days for vacuum-packaged meat. For reindeer meat packaged in MAP with CO_2 emitter (storage temperature 15 °C), lower TVCs were measured after 13 and 17 days (log 3 – 4 cfu/g) compared to MAP without emitter and vacuum-packaging at the same sampling times (log 4 – 5 cfu/g). Samples from the 3 packaging methods reached the same level of TVC (log 6 cfu/g) at the end of the storage time (day 21). The CO_2 level in the MA-packages with emitter displayed an initial decrease after 1 day of storage to 56%, but

increased to 67% towards the end of the storage time. The packaging strategy with CO_2 emitter also resulted in a significantly reduced drip loss for the reindeer meat; 1% for MAP with emitter compared to 3% for MAP without emitter. The article concluded that the capacity of the custom-made emitter was too low for the product, implying that the beneficial effect of an emitter could be expected to be more pronounced.

In a similar study by Holck and others (2014) where the emitter capacity was more carefully tuned towards the food product, the emitter compensated well for CO_2 absorbed by chicken fillets in MAP (100% CO_2) and the drip loss was drastically reduced; from a weight loss of 7.5% for fillets packaged without emitter, to 2.5% for fillets packaged with an emitter in MA-packages with the same g/p ratio (2.5). The effect was assumed to be due to a reduction in packaging collapse and physical squeeze on the fillet. The emitter maintained the CO_2 level close to 100% throughout the storage time, while for the MA-packages without emitter the CO_2 level dropped slightly to 90 – 95% depending on the g/p ratio. The microbial shelf life was found to be the same for fillets packaged in 100% CO_2 with emitter and in 100% CO_2 with regular fluid absorber. For the chicken packaged in 100% CO_2 with emitter, significant bacterial growth inhibition was detected; the bacteria required 7 additional days to reach a level of 10^7 cfu/cm^2 compared to packaging with commonly applied gas composition of 60% CO_2 and 40% N_2. This study reports that packaging in 100% CO_2 without emitter is not possible due to an unacceptably high drip loss.

Trindade and others (2013) evaluated the use of a combined oxygen scavenger and carbon dioxide emitter sachet (Didai, technology unknown) for active packaging of lamb cuts. The

study did not draw conclusions on a significantly prolonged shelf life for the product with emitter applied in vacuum-packages. The percentage level of CO_2 generated in the vacuum-packages including an O_2 scavenger/CO_2 emitter was reported to be 45% CO_2 after about 17 days of storage. The same CO_2 level was measured in the control vacuum-packages without emitter, speculated by the authors to be a result of CO_2 emission by anaerobic microorganisms. The CO_2 level is unexpectedly low for vacuum-packages with O_2 scavenger/CO_2 emitter, considering the limited available volume and that the O_2 level is stated to be zero. No information is provided on the total gas composition and the authors do not describe how the headspace gas composition in vacuum-packages was measured. Furthermore, it is not stated if the emitter capacity was product-adjusted and the concept of the technology is not clarified. In a different study, by Chen and Brody (2013), packaging with a CO_2-releasing packaging structure (CSP Technologies, technology unknown) was found to be effective at controlling the proliferation of artificially inoculated *Listeria monocytogenes*, as well as TVC and Enterobacteriaceae on a vacuum-packaged ready-to-eat meat product (cooked ham) stored at different temperatures and monitored over a 4-week period. The percentage levels of CO_2 gas in the vacuum-packages were measured as 56%, 72%, and 69% at 4, 10, and 22 °C, respectively, at the end of the storage period. Inoculation of *L. monocytogenes* is not an industrially relevant scenario as the focus is on preventing these bacteria from growing on the food product, rather than inhibiting the bacteria already present. Furthermore, interpretation of the results for this study is complicated by the lack of documented sampling data (bacterial counts) during the storage time. The 2 latter studies are not included in Table 6 as the underlying technologies (active substances) of the emitters have not been published.

Comparisons of different CO_2 emitter concepts are challenging. Differences in pre-handling of the food product, storage conditions and temperature, type of packaging material, g/p ratio, package size, and gas composition are all variables that will have an impact on the effect that the CO_2 emitter contributes to the shelf life of the food product. Another important factor concerning the evaluation of CO_2 emitter that is rarely documented is the material and density of the emitter substrate. The type and structure of the substrate in which the active ingredients are incorporated is of importance for liquid absorption and the amount and rate of CO_2 release. Examples of materials applied in different layers of CO_2 emitters/liquid absorbers are cellulosic fiber, or other fiber-based materials, SAP (super-absorbent polymer), other hydrogels and perforated plastic films. In addition, the active ingredients may be evenly distributed in pores of the substrate material or in a bulk deposit in the core of the substrate. These aspects should be taken into consideration when comparing the performance of different emitter systems.

The results of earlier scientific studies, as summarized in the previous paragraphs, have shown that an optimal effect of CO_2 emitters can only be achieved when the emitter capacity is optimized, that is adapted to the physiological properties and weight of the food product in question. An emitter with an optimal capacity will ensure an adequate CO_2 level, counteract formation of negative pressure within the package, ensure sufficient liquid absorption, and extend shelf life. Optimization of the emitter capacity was investigated in a study by Hansen and others (2009b) for salmon fillets in MA-packages with different fillet sizes and g/p ratios. A model was developed based on the results, making it possible to

calculate the required amounts of sodium bicarbonate and citric acid, based on weight and surface area of the salmon fillets, g/p ratio, and tray capacity. Additional research is required on this topic focused on product-specific concepts, making CO_2 emitter technologies more flexible and suitable for a broader range of food products.

With regard to commercialization, there are different emitters already on the market today. Emitters based on sodium bicarbonate and citric acid include CO_2 Freshpads (CO_2 Technologies, Urbandale, Iowa, USA)(Kerry 2014), SuperFresh (Vartdal Plastindustri AS, Vartdal, Norway), and the Active CO_2 pad (CellComb AB, Säffle, Sweden). In addition, UltraZap® XtendaPak (Paper Pak Industries, La Verne, CA, USA), and the CO_2Pad (McAirlaid's GmbH, Steinfurt, Germany) are based on other CO_2-releasing concepts.

Antimicrobial Packaging Systems

Antimicrobial food packaging presents a system designed to inhibit the growth of spoilage and pathogenic microorganisms. In this review, the most studied antimicrobial food packaging systems have been classified according to their active substance/material: essential oils (Table 7); enzymes and bacteriocins (Table 8); antimicrobial polymers (Table 9); and organic acids, their derivatives and other organic compounds (Table 10). Furthermore, antimicrobial nanoparticles are reviewed separately as the nano-size itself either increases or enables the antimicrobial activity (Table 11).

Essential Oils (EOs)

Recent interest in reducing the use of petroleum-based additives as active materials for food preservation has led to the application of natural additives both for the benefit of the individual as well as for the environment (Alves-Silva and others 2013). Essential oils (EOs) are secondary metabolites and play an important role in plant defense, thus, some of them possess strong antimicrobial properties. In addition, most of them are classified as GRAS (Ruiz-Navajas and others 2013) and, as a result, EOs have been extensively studied as additives in bio-based emulsified films and coatings. Many scientific publications are connected to the potential interest in this type of active packaging but without any real application to food. Some studies, however, have demonstrated the effectiveness of EO-enriched packages containing food and these are presented in Table 7.

Cinnamon essential oil (CEO) is among the most studied EOs in active materials. Gherardi and others (2016) showed that a multilayer material containing about 18 and 10% of cinnamaldehyde as the major compound of the selected EO showed high activity against *E. coli O157:H7* and *S. cerevisiae*, as the material reduced both microorganisms by 3 log CFU/mL. Compared to the results obtained in culture media, *E. coli* showed higher sensitivity to active materials in tomato puree. Interestingly, to maintain greater CEO in the film and avoid too much loss of volatile substances, an antimicrobial packaging material was developed by incorporating a cinnamon essential oil/β-cyclodextrin inclusion complex into polylactic acid nanofibers via an electrospinning technique (Wen and others 2016). Application in the preservation of pork (25 °C) showed that the sample packed with the nano-film decayed on the eighth day compared to the third day for the control packed with fresh-keeping film. In this study, the initial bacterial load of the pork was 10^3–10^4 CFU/g on

1294 the first day and the unpacked control pork reached an excessive number of colonies after 4
1295 days (above 1.10^7 CFU/g). Another option to control the release of major compounds of CEO
1296 is to reversibly anchor cinnamaldehyde to a polymer, such as chitosan films, via imino-
1297 covalent bonding (Higueras and others 2015). The antimicrobial properties of chitosan-Schiff
1298 base films in milk inoculated with *Listeria monocytogenes* led to a growth inhibition for 12
1299 days under refrigeration conditions.
1300
1301 Carvacrol is another EO regularly used as a bio-based bioactive compound. However, the
1302 synergistic antimicrobial effect of different EOs on food has been less frequently studied.
1303 One example is the study by Campos-Requena and others (2015) based on carvacrol and
1304 thymol, both included in HDPE/modified montmorillonite nanocomposite films. A synergistic
1305 antimicrobial effect was observed with *Botrytis cinerea*, when the films were applied
1306 through indirect contact with strawberries. The half maximal inhibitory concentration (IC50)
1307 of the EOs in the film was reduced from 40.4 mg/g (carvacrol only) to 13.2 mg/g (both EOs
1308 50:50). Knowing that the major volatile compounds of oregano EO is carvacrol, Rodriguez-
1309 Garcia and others (2016) evaluated the effect of oregano EO applied within pectin coatings
1310 on the inhibition of *Alternaria alternata* on tomatoes. The authors showed that 25.9 g/L was
1311 effective in inhibiting microbial growth.
1312
1313 To enhance safety and shelf life of cooked cured ham, Ruiz-Navajas and others (2015)
1314 studied 2 Spanish endemic species of thyme, *Thymus piperella* and *Thymus moroderi*. They
1315 reported that *T. piperella* had a higher effect than *T. moroderi*, probably due to the higher
1316 concentration of carvacrol in the former (predominant compound, 31,9%). Addition of both

EOs into films (from 1 to 2%) significantly decreased the aerobic mesophilic and lactic acid bacteria counts in food samples, with lowest counts for *T. piperella* at 2%. After 21 days, for example, the addition of 2% EOs led to a reduction of 0.87 and 0.53 log cycles of aerobic mesophilic bacteria compared to the uncoated samples of *T. piperella*- and *T. moroderi*-based films, respectively. In another study with thyme EOs, Quesada and others (2016) designed an active packaging system for the shelf-life extension of sliced ready-to-eat cooked pork during refrigerated storage. Interestingly, the package included an inner surface coated with a chitosan film with thyme essential oil (0%, 0.5%, 1%, and 2%) and was not in direct contact with the meat to avoid modification of organoleptic properties. The authors reported that yeast populations were affected by the presence of thyme EO and the yeast counts decreased as a function of the EO dose in the film, especially during the first 21 days of storage.

Arfat and others (2015) investigated microbiological and sensory changes of sea bass slices wrapped with fish protein/fish gelatin composite films incorporated with basil leaf essential oil (BEO) during storage at 4 °C for 12 days. Films were incorporated with 100% BEO (w/w, based on protein content). The shelf life was longer for samples wrapped with material incorporating BEOs (10-12 days) compared to the control (6 days). *Allium spp*. extract and vanillin have also been proposed as bioactive EOs. With the former, Llana-Ruiz-Cabello and others (2015) showed an efficiency against molds in lettuce during 7 days of storage (6.5% Proallium®). With the latter, Lee and others (2016) demonstrated that crab sticks packed with starfish gelatin films containing 0.05% vanillin exhibited antimicrobial activity against *L. monocytogenes* previously inoculated on the food product.

This brief overview illustrates that essential oil-based packaging has the potential to enhance food preservation. However, such packaging have not yet been extensively commercialized. Various factors need to be considered, such as the impact of EOs on (1) the organoleptic profile of the target food, (2) the physio-chemical properties of the materials, and (3) the effectiveness of this packaging system when manufactured under real conditions. In order to limit the constraint of their strong odor and taste, EO-based materials can be selectively used with compatible foods in terms of flavor. Another option could be the development of tasteless, colorless, and odorless EO derivatives (sensory inertness), such as some curcumin derivatives (Coma and others 2011; Etxabide and others 2016).

According to the recent scientific publication by Dornic and others (2016), although EOs contain compounds naturally produced in the natural environment by higher plants, their consumption may nevertheless present a risk to health, given their composition. Indeed, their consumption may cause adverse effects when used inappropriately. The recent study of Rivaroli and others (2016) showed that higher doses of 3.5 g/animal/day could have a pro-oxidant effect in feedlot livestock. As mentioned by Eghbaliferiz and Iranshahi (2016), natural antioxidants can act as pro-oxidants, which produce free radicals and cause DNA damage and mutagenesis. Consequently, further research is needed to understand the potential toxicity of EOs incorporated into packaging materials.

Enzymes and Bacteriocins

Incorporation of proteins, particularly enzymes and bacteriocins, into food packaging to control spoilage caused by food pathogenic microorganisms has been an area of research for several decades (Table 8).

Enzymes can serve as effective antimicrobials in food packaging by being chemically bonded to, or physically entrapped in, packaging films. As an antimicrobial enzyme, lysozyme can destroy the glycosidic bonds of the Gram-positive bacterial peptidoglycans. Lysozyme incorporated into whey protein films (204 mg/g of film) migrated into the food and inhibited the growth of *Listeria monocytogenes* to 4.4 log CFU/cm^2, extending the shelf life of smoked salmon (Min and others 2005). Barbiroli and others (2012) reported incorporation of lysozyme and lactoferrin into paper containing carboxymethyl cellulose, which allowed non-covalent binding of the positively charged proteins to the paper matrix. Tests on thin meat slices laid on paper sheets containing either or both antimicrobial proteins indicated that lysozyme was most effective in preventing growth of aerobic bacteria in the meat sample, giving almost 1 log cycle reduction with respect to the control. Lysozyme is accepted by the U.S. Food and Drug Administration (FDA 2001) as an antimicrobial agent in casings for frankfurters, and in Europe the use of lysozyme (E1105) falls under Directive 95/2/EC on food additives (European Union 1995).

Bacteriocins are peptides or small proteins, produced by some species of lactic acid bacteria (LAB), which inhibit the growth of food spoilage bacteria, mainly Gram-positive bacteria. The bacteriocin nisin has been successfully incorporated into methylcellulose/hydroxypropyl methylcellulose coatings (Franklin and others 2004) or PE films (Siragusa and others 1999), and

it has been coated on LDPE films (Mauriello and others 2005; Neetoo and others 2008) or paperboard (Lee and others 2004). Subsequently, the effective inhibition of bacterial growth was achieved in such foods as hot dogs, beef, milk, cold-smoked salmon, and orange juice. For example, packaging films coated with a cellulose derivatives-based solution containing 10,000 and 7,500 IU/mL nisin significantly decreased *Listeria monocytogenes* populations on the surface of hot dogs by greater than 2 log CFU per package after 60 days of refrigerated storage (Franklin and others 2004). Similarly, it was established that nisin-coated LDPE films were effective in inhibiting the bacterial flora in milk stored at 4 °C for 7 days, and the most significant results were observed in raw milk and pasteurized milk with a reduction of 0.9 and 1.3 log, respectively. Nisin (E234) has been authorized for food preservation in Europe under Directive 95/2/EC on food additives (European Union 1995).

The incorporation of nisin with other antimicrobial agents into PE and PE/polyethylene oxide films or polyamide coatings effectively inhibited *Brochothrix thermosphacta*, coliform bacteria growth and extended the shelf life of beef (Cutter and others 2001; Kim and others 2002) and fresh oysters (Kim and others 2002). According to the research of Khan and others (2016), the immobilization of nisin and EDTA on the surface of the cellulose nanocrystal/chitosan-films, by using genipin as a cross-linking agent, restricted the growth of psychrotrophs, mesophiles and *Lactobacillus* spp. in fresh pork loin meats, and increased the microbiological shelf life of the meat sample by more than 5 weeks. The films also reduced the counts of *E. coli* and *L. monocytogenes* in meat samples by 4.4 and 5.7 log CFU/g, respectively, after 35 days of storage. Furthermore, through the formation of nisin, citric acid, EDTA, and polyethylene glycol

sorbitan monooleate-coatings on polymeric films of different hydrophobicity (polyvinylchloride, nylon or linear LDPE), the shelf life of refrigerated broiler drumsticks was extended by 0.6 to 2.2 days (Natrajan and Sheldon 2000). In other studies, nisin, in combination with enterocins, sakacin, and potassium lactate, was incorporated into interleaves and tested on cooked ham and bacterial growth of *L. monocytogenes* (Jofré and others 2007) and *Salmonella* spp. (Jofré and others 2008) was successfully inhibited. Other bacteriocins such as enterocins (Marcos and others 2007), lactocins (Massani and others 2014), natamycin (De Oliveira and others 2007), and pediocin (Santiago-Silva and others 2009) have been incorporated into biopolymer-based films or used as coatings on various substrates, and they reduced bacterial (*L. monocytogenes*, *Lactobacillus plantarum*, *Listeria innocua*, *Salmonella* spp.) or fungal (*Penicillium roqueforti*) growth on cooked ham, Wieners (2.5 log reduction), Gorgonzola cheese, and sliced ham (0.5-2 log reduction), respectively.

Further research effort is still needed to evaluate the release of enzymes and bacteriocins from various films and coatings into packaging, as well as diffusion to the surface of the food. Moreover, the impact of the packaging on sensory properties of food should be thoroughly assessed. To date, only nisin and natamycin have been approved for use as food additives in various countries including the USA and the EU. Therefore, legislative issues regarding the use of bacteriocins as food preservatives remain the main limitation in their commercial exploitation. Nevertheless, the use of enzymes and bacteriocins in combination with other preservation techniques can produce synergistic effects in food packaging while maintaining the safety and quality of minimally processed and fresh food products.

Antimicrobial Polymers

Some polymers like chitosan or ε-polylysine are inherently antimicrobial and are used in films and coatings (Table 9). ε-Polylysine is a natural antimicrobial polypeptide that is effective against Gram-positive and Gram-negative bacteria. However, only a few studies have reported on polylysine incorporation into packaging materials. For example, Zinoviadou and others (2010) developed ε-polylysine-containing whey protein films that significantly reduced the specific growth rate of total flora and completely inhibited lactic acid bacteria growth in fresh-cut beef portions as well as prolonged shelf life.

Chitosan, along with its derivative products (such as chitooligosaccharides), presents antimicrobial and antifungal activity against a wide range of target microorganisms, and it has also been proven to be beneficial to food packaging. Chitosan has been incorporated as an antimicrobial additive into food packaging with synthetic polymers such as LDPE (Park and others 2010) and bio-based polymers such as carboxymethylcellulose (Youssef and others 2016) or used as a coating on plastic films (Joerger and others 2009). When chitosan-incorporated LDPE films were applied on fresh sliced red meats, microorganisms on the meat surface were not inhibited but significant extension of red color shelf life was observed in refrigerated samples (Park and others 2010). Meanwhile, bio-nanocomposite films containing chitosan had an effect on the total bacterial counts, mold and yeast counts, and coliforms in soft white cheese during 30 days of storage at 7 °C, and increased its shelf life (Youssef and others 2016). Total bacterial counts significantly decreased during storage especially for samples that were

coated with preservative films. Furthermore, the coliform, mold and yeast organisms in soft cheese were inhibited by the active films. Moreover, ethylene copolymer film was coated with chitosan through attachment of the polymer to the corona-treated surface of the film, and the antimicrobial activity of the composite film against *Listeria monocytogenes Scott A* was tested on turkey breast and a log reduction of about 1.7 after 10 days and 1.2 after 15 days at 4 °C was achieved (Joerger and others 2009).

Ye and others (2008a) determined that chitosan-coated plastic films were not able to control the growth of *L. monocytogenes* on ham steaks and, therefore, evaluated the antilisterial efficacy of chitosan-coated plastic films incorporating 5 additional GRAS antimicrobials: nisin, sodium lactate, sodium acetate, potassium sorbate, and sodium benzoate. The incorporation of those antimicrobials into chitosan-coated plastic film retarded or inhibited the growth of *L. monocytogenes*, while the film containing sodium lactate was the most effective antimicrobial film and showed excellent long-term antilisterial effect with the counts of *L. monocytogenes* being slightly lower than the initial inoculum. Similarly, the same films inhibited the growth of *L. monocytogenes* on cold-smoked salmon samples for at least 6 weeks (Ye and others 2008b). However, the authors indicated that sensory studies are needed before this technology is further developed (Ye and others 2008a).

The antimicrobial activity of chitosan (low molecular weight, 150 kDa, 75-85% deacetylation) coating with 5-10% of lauric arginate ester (LAE), 2-20% of sodium lactate, and 0,3-0,6% of sorbic acid (alone or in combination) on PLA films was verified using *Listeria innocua* and

Salmonella typhimurium (Guo and others 2014). Most effective combinations were 5% chitosan/5% LAE/2% sodium lactate/0.3% sorbic acid and 5% chitosan/5% LAE. Both combinations reduced *S. typhimurium* to an undetectable level at 0, 24, and 48 h, and significantly reduced *L. innocua* (even 6 logs after 48 h). Antimicrobial tests on surface-contaminated turkey slices led to the reduction of *L. innocua* growth by 3 log CFU/cm^2 for both films. The films also reduced the growth of *L. monocytogenes* on the surface of ready-to-eat meat by 2.5-3 log CFU/cm^2 during storage of 3 or 5 weeks at 10 °C. For *S. typhimurium* the reduction was 1.5 log CFU/cm^2.

The effect of chitosan used in combination with nisin, potassium sorbate, or silver-substituted zeolite incorporated into LDPE on the physicochemical and microbial quality of chicken drumsticks stored at 5 °C for 6 days was also investigated (Soysal and others 2015). Total aerobic mesophilic bacteria counts of samples packed in bags containing 2% of chitosan, nisin, zeolite, and potassium sorbate in LDPE layer were 1.03, 0.98, 0.51, and 0.17 times lower, respectively, than those of samples packed in control bags. Moreover, samples packed in active bags had lower TBARS values than those of samples in control bags. The exploitation of GRAS antimicrobials nisin and potassium sorbate in food packaging is straightforward, whereas the use of silver zeolite as a surface biocide is debatable. Although it is approved by the US FDA as a food contact substance, in the EU it is not included in the list of authorized substances, but is in the provisional list for use in accordance with national law. In another study, chitosan films were developed by incorporating lauric arginate ester (LAE) and their antimicrobial activity against mesophiles, psychrophiles, *Pseudomonas spp*., coliforms, lactic acid bacteria, hydrogen

sulfide-producing bacteria, yeast and fungi was evaluated on chicken breast fillets at 2, 6, and 8 days (Higueras and others 2013). Chitosan films demonstrated antimicrobial activity in the range of 0.47-2.96 log reduction, dependent on time and bacterial group studied, while the incorporation of 5% LAE in the film increased antimicrobial activity to 1.78-5.81 log reduction.

It should be noted that chitosan has been given GRAS status by the U.S. FDA (FDA 2002, 2005, 2011) for agricultural and medicinal purposes, but it is not yet specifically approved as an antimicrobial food additive. Meanwhile, the other antimicrobial polymer mentioned above, polylysine, was granted GRAS status by the U.S. FDA in 2004 (FDA 2004). Along with excellent antimicrobial properties, packaging coatings and films prepared from such biopolymers exhibit a variety of other advantages, such as biodegradability, edibility, nontoxicity, biocompatibility, an aesthetic appearance, and good barrier properties. However, further studies are needed to fully evaluate industrial feasibility and the commercial viability of implementation of the proposed technologies. Furthermore, there is a need to evaluate the packaging effects on the sensory properties of food as well as to validate already developed packaging by using commercial food products held under real storage conditions.

Organic Acids, their Derivatives and other Organic Compounds

Some organic compounds such as selected organic acids and their derivatives, exhibit antimicrobial activity (Table 10) and can be incorporated into packaging films.

Citric Acid. Júnior and others (2015) investigated the antimicrobial activity of citric acid on packaged minced beef. 30% of a mixture of citric acid/cornstarch/glycerol (ratio: 1.5:68.5:30) was incorporated in extruded cornstarch/LLDPE films. Although the microbial population increased in all the samples, less growth was observed in minced beef packed with active films compared to the control samples at the end of a 10-day evaluation period. The authors reported a reduction in total bacteria counts of approximately 1 log CFU/g. The results identified the potential of active films containing citric acid to extend the shelf life of minced beef. However, further research needs to be conducted to improve the limited antimicrobial effect demonstrated in this study.

Sorbic Acid. García-Soto and others (2015) incorporated 0.5% and 1% of sorbic acid and 8% of algal extract (*Fucus spiralis*) into PLA films to protect the flat fish megrim (*Lepidorhombus whiffiagonis*) from microbial growth. The authors reported a positive antimicrobial effect against psychrotrophs with a reduction level of 0.9 log CFU/g in comparison to PE films and lower mean values for aerobes and Enterobacteriaceae after 7 days of storage. Although the results obtained do not demonstrate a significant antimicrobial effect at the end of the shelf life (11 days), improved sensory properties (external odor, gill appearance, and odor) were reported for megrim packed with active films, while control samples were considered unacceptable from a sensorial point of view. Limjaroen and others (2005) incorporated sorbic acid into solvent cast poly(vinylidene chloride) (PVDC) films. Beef bologna and cheddar cheese, inoculated with *L. monocytogenes* (10^3 and 10^5 CFU/g each), were wrapped in PVDC films containing 1.5 or 3% w/v sorbic acid. After 28 days of storage at 4 °C, lower *L. monocytogenes*

counts were obtained in beef bologna samples packed with active films and inoculated with 10^5 CFU/g (4.4 log lower for both sorbic acid films, compared to the control). In the inoculated cheddar cheese samples, the active films did neither significantly affected the growth of the inoculated *L. monocytogenes* nor that of mesophilic aerobic bacteria after 35 days of storage at 4 °C. Beef samples inoculated with 10^3 CFU/g, in contrast, demonstrated 6.5 and 7.2 log lower *L. monocytogenes* counts for 1.5 and 3% sorbic acid films, respectively, compared to the control. Moreover, mesophilic aerobic bacteria and LAB counts in the beef packages with the active films were found to be around 4 and 6 log lower than in control samples with initial *L. monocytogenes* inoculums of 10^5 and 10^3 CFU/g, respectively. This research, however, has some drawbacks, especially because the use of sorbic acid as an additive in meat products is restricted according to Commission Regulation (EU) No 1129/2011 (European Commission 2011). Sorbic acid can only be used for selected applications for meat products, such as aspic, pate, and surface treatment of dried meat products, jelly coatings, and collagen-based casings of meat products at the maximum level of 1 g/kg or *quantum satis*. Moreover, the reported sample preparation, a solution-casting, laboratory-scale method, cannot be applied on an industrial scale. This trial should be repeated using melt (extrusion), however, the high temperature may influence the sorbic acid activity.

Potassium sorbate. Cestari and others (2015) developed thermoplastic starch/PBAT blended films with 5% potassium sorbate content to prevent microbial growth in restructured chicken steaks during frozen storage. After 30 days of storage, *Escherichia coli* (initial count 1.94 log CFU/g) was not detected in samples packed with potassium sorbate films, while *E. coli* was

detected in control samples (1.3 log CFU/g). In chicken steaks packed with the active films, *E. coli* was kept under the detection limit until completion of frozen storage (150 days). Kaya and others (2015) reported the use of potassium sorbate and/or sodium lactate (3% applied alone and 1.5% of each, applied in combination) in brine to protect smoked rainbow trout (*Oncorhynchus mykiss*) fillets against microbial growth. The trout fillets were kept in 8% NaCl brine for 12 h before smoking. After 4 weeks of storage at 6 ±1 °C, total aerobic mesophilic bacteria counts were shown to be about 3, 2.1 and 1.7 log CFU/g lower for trout kept in potassium sorbate, sodium lactate and its combination, respectively, compared to the control (about 8.2 CFU/g) kept in brine without preservatives. Similar results were observed for yeast and molds. Additionally, after 5 weeks of storage an identification of the bacteria species in the applied samples was performed. With the exception of the trout kept in potassium sorbate brine, *Serratia liquefaciens,* which is considered as one of the main pathogens and spoilage bacteria in smoked fish, was the dominating species. This indicates that potassium sorbate was effective against this pathogen as it was not present in the corresponding samples.

Potassium metabisulfite. Several fruits and vegetables are highly susceptible to enzymatic changes, and the application of some antimicrobials can provide additional properties against this problem. Foralosso and others (2014) tested PVC films that contained a 0.1, 1, or 2% w/w mixture of pure and encapsulated potassium metabisulfite (ratio 1:1) as an active (antimicrobial, antioxidant and antibrowning) substance. Cut Gala apples (*Malus domestica*) were wrapped in the active PVC films, and stored at 4, 8, 12, 16, and 20 °C and 30% RH. Samples wrapped in PVC films with 1 and 2% potassium metabisulfite mixtures resulted in a

lower browning index which was rated to be around 60 and 50%, respectively, compared to the control (around 90%), and a shelf-life extension from 4 to 8 days for apples stored at 8 °C was reported. Samples wrapped using 2% potassium metabisulfite mixtures and stored at 4 °C demonstrated toxicological and microbiological stability (migration of sulfites below 10 mg/kg SO_2, according to Brazilian regulation for plastic materials in contact with food; and microbial counts below 10^6 CFU/g, considered as the quality threshold by the authors) throughout the 20-day storage period. The active film provided the conditions suitable for apple consumption up to 12 days of storage at 8 and 12 °C complying with the microbiological contamination limit of 6 logs CFU/g.

Oxidized regenerated cellulose. Sezer and others (2016) incorporated oxidized regenerated cellulose micro-particles (4% w/w) in poly(Ɛ-caprolactone (PCL) films and evaluated their antibacterial activity on packed sliced salami inoculated with *L. monocytogenes* (10^4 CFU/g). After 14 days of storage at 4 °C in contact with the active PCL films, 50% of total colony-forming units (about 8 log CFU/g) of *L. monocytogenes* did not survive. The packaging also led to a decrease in the growth of *E. coli* and *S. aureus*. Moreover, active films containing 4% of oxidized regenerated cellulose micro-particles reduced the oxygen and water permeability by 93 and 70%, respectively.

Allyl isothiocyanate. A high antibacterial activity is reported for allyl isothiocyanate (AITC) against a wide range of bacteria (Kim and others 2015). Pang and others (2013) reported the positive effect of using AITC (18 and 36 µg/L) in the vapor phase when applied, alone and in

combination with MAP (49% CO_2/0.5% O_2/50.5% N_2), to catfish fillets stored at different temperatures. The authors observed that AITC (alone or in combination with MAP) had an antimicrobial effect against *Pseudomonas aeruginosa* and extended the shelf life of fresh catfish fillets from 4 to 5 days (18 µg AITC/L), 11 (36 µg AITC /L) and 23 days (MAP combined with both concentrations of AITC) at 8 °C. The latter applications maintained the *P. aeruginosa* counts at a level of about 3 CFU/g during 23 days, compared to the control without MAP (about 9 log CFU/g after 7 days) and with MAP (about 7.5 log CFU/g after 12 days). At 15 and 20 °C, the combination of both technologies was not as effective as at 8 °C, but still extended the shelf life at least 2.6 times compared to the controls. No sensory analysis of catfish fillets was performed at the end of storage. However, due to the pungency and strong smell of AITC, a sensory analysis of the final product should be performed to assure the acceptability of the product. In the context of odor, Kim and others (2015) recommend the application of AITC in vapor phase and low concentration (0.02-2500 mg/mL) to avoid negative impact on food.

Commercial packaging solutions containing AITC can be found in a variety of formats (sheets, labels and films) on the Japanese market under the trademark Wasaouro™ (Mitsubishi-Kagaku Foods Corporation, 2002). However, even though antimicrobial tests with AITC (occurring in mustard) were successful on several types of food products (Kim and others 2015) and it has been given GRAS status (FDA 2006), it has to be emphasized that the regulations in specific countries can differ and data regarding current status can change (such as the approval status for AITC in EU and USA). In 2010, the EFSA panel on food additives and nutrient sources added to food (ANS) gave its scientific opinion on the safety of allyl isothiocyanate for the proposed

uses as a food additive. Therein, it is stated that AITC is "*an efficient alternative to already approved preservation techniques for a range of foods*," such as bakery products (including all types of pre-packed bread and fine bakery ware), all types of cheese, fruits, and vegetables (EFSA ANS Panel, 2010). To give another example, for sorbic acid, and its derivatives, such as potassium sorbate, the EFSA has re-evaluated their status as food additives in 2015 (EFSA ANS Panel 2015). The main hurdle to commercialization of active packaging solutions containing organic compounds are regulatory requirements. Therefore, research efforts should be focused on the development of tailor-made active packaging solutions that comply with the specific legislation for each food product.

Nanoparticles

Antimicrobial nanomaterials represent an increasingly important component of some active packaging for food applications (Ayhan 2013). Antimicrobial nanoparticles (particles between 1-100 nm in size) are incorporated into a polymer matrix with the aim of prolonging the shelf life of packaged food. High surface-to-volume ratio and enhanced surface reactivity of the nano-sized antimicrobial agents cause inactivation of microorganisms more effectively than their micro or macro-scale counterparts (Radusin and others 2016). The preparation of food packaging materials depends on the nature of the nanoparticle, its size, and its specific surface area.

Despite the large number of studies reported in the literature in this area, there are only a few studies incorporating real food systems. Commonly used or tested antimicrobial nanoparticles

are metal ions (silver, copper, gold, platinum), metal oxide (titanium dioxide, zinc oxide, magnesium oxide), and organically modified nano-clays. From ancient times, silver (Ag) has been used as an antimicrobial agent. Its ability as an antimicrobial agent increases in nano-dimension and, hence, there are now many studies with Ag nanoparticles incorporated in food packaging materials as antimicrobial agents (Panea and others 2014; Azlin-Hasim and others 2016; Li and others 2017) (Table 11). The most recent studies illustrate that addition of Ag nanoparticles into different polymer matrices, in combination with other additives or nanoparticles, can significantly prolong the shelf life of different foodstuffs. Li and others (2017) reported that rice stored in LDPE without Ag/TiO_2 showed a serious mildew condition after one month with increased total plate counts (TPC) from 4.84 to 7.15 log cfu/g, while the rice stored in a nanocomposite based on LDPE with Ag/TiO_2 had a low TPC of 5.48 log cfu/g. Mihaly Cozmuta and others (2015) reported that the microbiological safety of bread stored in Ag/TiO_2-based packaging inhibited the proliferation of yeast/molds, *B. cereus*, *and B. subtilis.* The shelf life of bread was extended by reducing the degradation rate of the main nutritional compounds compared to the bread stored in an open atmosphere or in a commonly used plastic packaging. Azlin-Hasim and others (2016) prepared nanocomposite material based on PVC and silver nanoparticles, and they reported that this significantly extended the product shelf life and resulted in lower lipid oxidation of chicken breast fillets, while Panea and others (2014) reported reduction in MO but with higher lipid oxidation.

Emamifar and others (2010) conducted a study on the antimicrobial activity of LDPE loaded with nano-silver and zinc oxide (ZnO) for the packaging of orange juice. This system was very

effective in prolonging the shelf life of orange juice (up to 28 days). ZnO has also been used as an antimicrobial agent added to active packaging films for packaging fresh poultry meat by Akbar and Anal (2014), and they showed a reduction of the initial bacterial counts (*S. aureus* and *S. typhimurium*) by 2 log within 24h of incubation at 8±1 °C. After 6 days there were no viable cells of *S. aureus,* and no *S. typhimurium* after 8 days of incubation.

Titanium dioxide (TiO_2) has been studied as antimicrobial nanoparticles in LDPE for the packaging of fresh pears, and a decrease in mesophilic bacteria from 3.14 to less than 2 log CFU/g for the entire storage period (17 days) was recorded, whereas for neat LDPE cell loads increased from 3.19 to 4.02 log CFU/g. Furthermore, yeasts decreased from 2.45 to less than 2 log CFU/g, whereas those for the control sample increased from 2.1 to 3.37 log CFU/g (Bodaghi and others 2013). In addition, copper (Cu) was effective against *Pseudomonas* spp. (isolated from spoiled fiordilatte cheese) when incorporated in PLA and used for packaging of fiordilatte cheese. A delay in microbial proliferation was recorded when the active films were used (Conte and others 2013).

As reported in the previous sections, the use of antimicrobial nanoparticles has great potential in preserving the microbial quality of the food systems. In this context, the appropriate antimicrobial agent needs to be selected according to the targeted food. Additionally, the impact of nanoparticles on the properties of the packaging films, such as barrier properties and transparency, should also be considered. However, the safety evaluation and approval for use of such nanoparticles in food packaging remains the greatest challenge due to the difficulties in

the evaluation of the safety of nanoparticles in general (Radusin and others 2016) as well as constraints associated with the current legislative landscape (Amenta and others 2015, Radusin and others 2016, Rauscher and others 2017).

Over the last decade, various studies have been conducted in this area and several scientific reviews have been published. However, these have mostly focused on technology and several mechanisms, as well as *"in vitro"* studies on culture media. There has been little research involving real food packaging systems. Such research, however, is of great importance, since the antimicrobial activity of the active agents with culture media does not necessarily correlate with the antimicrobial activity in the food. This is mainly due to the complex structure of the food as well as the differences in the antimicrobial activity test conditions.

Before an antimicrobial food packaging can be successfully developed, a number of factors have to be considered. Firstly, the food system has to be fully understood in terms of its components, and physical and chemical characteristics, such as pH, and water activity, as well as its microbiological aspects, including identification of those microorganisms that are desirable and undesirable. A suitable antimicrobial active agent should be selected with respect to all these characteristics. In particular, the antimicrobial spectrum and the efficiency of the agent should target the microorganisms that limit the shelf life of the particular food. According to the international standard on the measurement of antibacterial activity of plastics and other non-porous surfaces (ISO 22196 2011), derived from Japanese Industrial Standard (JIS Z 2801 2000), a decrease of the number of microorganisms in the magnitude of 2 log colony forming

units (CFU)/cm^2 is required to demonstrate antimicrobial efficacy. In food systems, shelf-life tests have to be performed to evaluate the efficiency of the antimicrobial film for the selected product. In this context, the maximum permitted level of microorganisms in a food is very specific and depends on several factors, such as the type of microorganisms (spoilage or pathogenic), the type of food and the regulations in force in the country where the product will be marketed. In the EU, for instance, the microbiological criteria for foodstuffs are regulated by the Commission Regulation (EC) No 2073/2005) (European Commission 2005). For some food systems, such as several bakery products, no visual mold growth should be observed, whereas for others, the number of microorganisms should not exceed a certain number. Additionally, the influence of the food on the efficiency of the antimicrobial agent should be considered since the agent may be entrapped or deactivated by the food component, or the activity of the agent may be affected by a low or high pH.

A second consideration is the storage conditions of the packed food since the temperature or relative humidity may affect the release and/or the efficiency of the active agent. A third factor involves selection of antimicrobial agents that do not cause any undesired changes in the food, such as the sensory properties. The last aspect to consider is that the addition of antimicrobial agents should not result in undesirable changes in the packaging material, such as barrier, sealing and adhesion properties, transparency, or glossiness, and it should not cause any increase in the migration of substances from the packaging material to the food.

Conclusion

Extensive research on the development of new active packaging technologies has been conducted over recent years generating a wide variety of active packaging systems that may be applied to extend the shelf life of food products. This review highlights the huge potential of active packaging systems and concludes that challenges in the implementation of new technologies to real food applications are similar across all the active packaging technology categories discussed. Food products are very complex systems and packaging parameters are highly product-specific. Thus, to achieve an optimal activity or capacity of the desired active packaging system, product-tailored concepts have to be applied. Thereby, it is crucial to consider all the influencing factors, such as the physical/chemical/physiological properties of the food, packaging size, and storage conditions. Scale up and industrialization of the active packaging technologies could be challenging and therefore should be taken into consideration at early development state for successful commercialization. The cost of the implementation of the technology has to correspond with the benefit gained by the particular food product, legislative and regulatory issues must be addressed, and broad consumer acceptance is required. A successful collaboration between research institutes and industry, including development, legislative and commercial functions, is required to overcome these challenges. However, the recent advances discussed in this review can provide food and packaging scientists with a better understanding of the potential and the benefits of active packaging technologies and, hence, assist in accelerating their commercial adoption.

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Author Contributions

The authors made the following contributions to the manuscript:

Selcuk Yildirim: Corresponding author. Development of concept of manuscript and distribution of work, interim and final revision, Abstract, Introduction, Conclusion, Oxygen scavengers,

Bettina Röcker: Coordination of authors’ contributions. Abstract, Introduction, Conclusion, Oxygen scavengers, Moisture scavengers, References

Marit Kvalvåg Pettersen: Carbon dioxide emitters

Julie Nilsen-Nygaard: Carbon dioxide emitters, Moisture scavengers

Zehra Ayhan: Ethylene absorbers

Ramune Rutkaite: Antioxidant releasers; Antimicrobial packaging systems (enzymes and bacteriocins, polymers)

Tanja Radusin: Antimicrobial packaging systems (nanoparticles)

Patrycyja Suminska: Moisture scavengers; Antimicrobial packaging systems (organic acids, their derivatives and other organic compounds)

Begonya Marcos: Antioxidant releasers, Moisture scavengers, Antimicrobial packaging systems (organic acids, their derivatives and other organic compounds)

Veronique Coma: Antimicrobial packaging systems (essential oils)

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Tables

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