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30	Word count of text: 28,810 words
31	
32	Short version of title: Active Packaging Applications for Food
33	
34	Choice of journal/section: Comprehensive Reviews in Food Science and Food Safety
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39 ABSTRACT

The traditional role of food packaging is continuing to evolve in response to changing market 40 needs. Current drivers such as consumer's demand for safer, "healthier," and higher-quality 41 foods, ideally with a long shelf-life; the demand for convenient and transparent packaging, 42 and the preference for more sustainable packaging materials, have led to the development 43 44 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 45 components that would release or absorb substances into or from the packaged food or the 46 environment surrounding the food." Active packaging materials are thereby "intended to 47 extend the shelf-life or to maintain or improve the condition of packaged food". Although 48 extensive research on AP technologies is being undertaken, many of these technologies have 49 not yet been implemented successfully in commercial food packaging systems. Broad 50 communication of their benefits in food product applications will facilitate the successful 51 development and market introduction. In this review, an overview of AP technologies, such 52 as antimicrobial, antioxidant or carbon dioxide-releasing systems, and systems absorbing 53 oxygen, moisture or ethylene, is provided, and, in particular, scientific publications 54 55 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 56 57 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 58 when applied to specific foods and hence can assist in accelerating commercial adoption. 59

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- 61 **Keywords:** active packaging, oxygen scavenger, antimicrobial packaging, antioxidant
- 62 releaser, ethylene absorber.
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- 64 END PAGE 2
- 65

# 66 Nomenclature

67	AA	ascorbic acid
68	AITC	allyl isothiocyanate
69	AnV	<i>p</i> -anisidine value
70	AP	active packaging
71	BEO	basil leaf essential oil
72	BHA	butylated hydroxyanisole
73	BHT	butylated hydroxytoluene
74	BI	browning index
75	CEO	cinnamon essential oil
76	CFU	colony-forming units
77	СРР	cast polypropylene
78	CSP	catalytic system with palladium
79	EDTA	ethylenediaminetetraacetic acid
80	EO	essential oil
81	EVOH	ethylene vinyl alcohol
82	EFSA	European Food Safety Authority
83	FFA	free fatty acids
84	GC	gas chromatography
85	GRAS	generally regarded as safe
86	HDPE	high-density polyethylene
87	IC	inhibitory concentration
88	IU	international units

89	LAB	lactic acid bacteria
90	LAE	ethyl-N <sup><math>\alpha</math></sup> -dodecanoyl- <i>L</i> -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	МО	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)

112	PVDC	poly(vinylidene chloride)
113	RH	relative humidity
114	SAP	super-absorbent polymer
115	SiO <sub>x</sub>	silicon oxide
116	SP/IP	smart and intelligent packaging
117	ТВА	thiobarbituric acid
118	TBARS	thiobarbituric acid-reactive substances
119	тсс	total coliform counts
120	TiO <sub>2</sub>	titanium dioxide
121	TMA	trimethylamine
122	TVC	total viable counts
123	(U.S.)FDA	(United States) Food and Drug Administration
124	WVTR	water vapor transmission rate
125	ZnO	zinc oxide
126		

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## 128 Introduction

129 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, 130 preventing any physical damage, and protecting against manipulation and theft. Packaging 131 132 also meets the fundamental need to maintain food quality and safety from production to 133 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 134 oxygen, moisture, light, dust, pests, volatiles, and both chemical and microbiological 135 contamination (Coles and others 2003; Yildirim 2011; Arvanitoyannis and Oikonomou 2012; 136 137 Pereira de Abreu and others 2012). The protective role of the packaging is primarily passive, 138 acting as a barrier between the food, the atmosphere surrounding the food, and the 139 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 140 exchange through the packaging (Lee and Paik 1997; Hussein and others 2015). Such 141 142 packaging systems, however, are limited in their ability to further extend the shelf-life of the 143 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-144 145 quality foods, which are non-processed or minimally processed, do not contain 146 preservatives, but offer an acceptable shelf-life (Singh and others 2011; Gerez and others 147 2013). In response, the protective function of packaging has been refined and improved 148 leading to the development of new packaging technologies, such as modified atmosphere packaging (MAP) (Ohlsson and Bengtsson 2002; Rodriguez-Aguilera and Oliveira 2009; 149

150 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 151 152 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 153 and Butler 2008; Lee and Rahman 2014; Realini and Marcos 2014; Biji and others 2015; 154 155 Brockgreitens and Abbas 2016), and the application of nanomaterials (Imran and others 156 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 157 packaging. 158

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Active packaging is an innovative approach to maintain or prolong the shelf-life of food 160 161 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 162 with the food in such a way as to "deliberately incorporate components that would release or 163 absorb substances into or from the packaged food or the environment surrounding the food" 164 165 (European Commission 2009). Active packaging systems can be divided into active 166 scavenging systems (absorbers) and active-releasing systems (emitters). Whereas the former remove undesired compounds from the food or its environment, for example, moisture, 167 168 carbon dioxide, oxygen, ethylene, or odor, the latter add compounds to the packaged food 169 or into the headspace, such as antimicrobial compounds, carbon dioxide, antioxidants, 170 flavors, ethylene or ethanol. Table 1 provides an overview of the primary active packaging 171 technologies and their potential benefits in food applications.

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173 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 174 175 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 176 of the food. Furthermore, the activity of the active substances when directly added to food 177 may be reduced or inhibited as a result of interaction between the active substances and the 178 179 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 180 of the food. 181

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A large variety of active packaging systems has been developed and, to date, numerous 183 184 reviews have emphasized the potential of active packaging technologies to supply safer, "healthier", and higher-quality foods to the consumer (Kuorwel and others 2015; 185 Brockgreitens and Abbas 2016). However, the number of reviews presenting the benefits of 186 active packaging technologies applied to specific food products is limited (Llorens and others 187 188 2012; Pereira de Abreu and others 2012; Cichello 2015). Active packaging technologies that 189 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. 190 191 For example, the release rates, absorption rates, or diffusion rates of active substances may 192 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 193 194 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.

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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.

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## 205 Oxygen Scavengers

The application of oxygen scavengers (OS) is one of the main active packaging technologies 206 that aims to remove any residual oxygen present in the food package (Solovyov 2010; 207 Arvanitoyannis and Oikonomou 2012; Realini and Marcos 2014) or improve barrier 208 properties by acting as an active barrier (Sängerlaub and others 2013a). Several food 209 210 products are sensitive to oxygen, thus, the food industry seeks to exclude oxygen from food 211 packaging. This is mainly achieved using gas flushing or modified atmosphere packaging processes. However, the residual oxygen-concentration in the package often remains 212 213 between 0.5-5% (Solovyov 2010; Gibis and Rieblinger 2011; Pereira de Abreu and others 214 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 215 216 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).

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Oxygen in packaging negatively affects the quality and shelf-life of several foods as it leads to 220 oxidation of the product (Choe and Min 2006) or promotes the growth of aerobic 221 microorganisms (Lee 2010; Solovyov 2010) resulting in color modifications (Møller and 222 223 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 224 2009a; Li and others 2013), or nutritional losses (Chung and others 2004; Lopez-Gomez and 225 Ros-Chumillas 2010; Van Bree and others 2012). A reduction in the residual oxygen level of a 226 227 food packaging can be achieved through the application of oxygen scavengers, in some cases 228 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 229 2010; Gibis and Rieblinger 2011; Polyakov and Miltz 2016), of which the OS activity is 230 triggered by moisture so that the reduced iron is irreversibly oxidized to a stable ferric oxide 231 232 trihydrate complex (Solovyov 2010). In contrast, other applied metals, such as cobalt (Galdi 233 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. 234 235 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), 236 ascorbic acid (Matche and others 2011; Pereira de Abreu and others 2012), gallic acid 237 238 (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 239

240 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 241 242 2014), or biological approaches using bacterial spores (Anthierens and others 2011), or veasts that are immobilized in a solid material (Edens and others 1992). Some commercially 243 available oxygen-scavenging solutions are SHELFPLUS® O2 (OS-masterbatch, Albis Plastic 244 GmbH, DE), AMOSORB<sup>™</sup> ColorMatrix<sup>™</sup> (different OS-solutions, PolyOne<sup>™</sup>, Europe Ltd., UK), 245 Cryovac<sup>®</sup> (OS-film, Sealed Air Corporation, USA), AGELESS OMAC<sup>®</sup> (OS-film, Mitsubishi Gas 246 Chemical Inc., USA), OxyRx<sup>®</sup> (OS-containers, Mullinix Packages Inc., USA), or Aegis<sup>®</sup> OXCE 247 (OS-masterbatch, Honeywell International Inc., USA). 248

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Much published work about oxygen-scavenging technologies suggests they have great 250 potential in food applications. However, such research has mainly been performed using 251 oxygen-scavenging sachets containing iron powder (Charles and others 2003; Solovyov 2010; 252 Antunez and others 2012; Cruz and others 2012; Kartal and others 2012; Chounou and 253 others 2013; Cichello 2015). In contrast to Asia or the USA, sachet-based applications are not 254 255 well accepted by consumers in European countries (Restuccia and others 2010), as they are 256 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 257 of such sachet-based active packaging technologies. Further drawbacks include the 258 requirement of an additional packaging operation step or their unsuitability in combination 259 with beverages or moist foods due to moisture sensitivity (Suppakul and others 2003; Day 260 261 2008; Pereira de Abreu and others 2012). Alternatively, several new oxygen-scavenging technologies have been developed over the last decade, such as incorporating active 262

substances directly into packaging films or containers. However, only few of them have been
successfully implemented in real food systems. Consequently, studies demonstrating the
benefits of alternative oxygen-scavenging systems to particular food products are rather
rare. Table 2 provides an overview of several oxygen-scavenging technologies which have
already been applied to food products.

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Iron. Shin and others (2009) applied an iron-based OS packaging to extend the shelf-life of processed meat products. Meatballs were packed in active PP-based multilayer trays containing OS materials (40, 80 and 100% w/w) in the middle layer. During a storage time of up to 9 months at 23 and 30 °C, oxidative-induced color and flavor changes of the meatballs packaged in the active OS containers (100% w/w) were significantly lower compared to those in the passive packages. This was also confirmed by TBA values (thiobarbituric acid) indicating less lipid peroxidation of meatballs in these OS packages.

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Military rations constitute a range of products in which a long shelf-life is of particular 277 278 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 challenge, especially with components with high oil content that are susceptible to oxidative 280 281 deterioration. Within this context, Gomes and others (2009) investigated the influence of an iron-based OS-containing laminate material for its ability to extend the shelf-life of a hot-282 filled meal-ready-to-eat (MRE) cheese spread, a component of military operational rations. 283 284 The authors demonstrated that the proposed  $O_2$ -absorbing laminate reduced the initial headspace oxygen concentration in MRE pouches from 20.4 to 6.82 vol.-% (67.44 vol.-% 285

286 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 287 288 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, 289 however, was assigned to the oxidative degrading reactions of the food. After 1 year of 290 storage, the positive effect of the O<sub>2</sub>-absorbing laminate was illustrated through MRE cheese 291 292 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<sub>2</sub>-293 absorbing laminate could be better preserved resulting in an almost 1.5 times higher 294 vitamin C content compared to the control samples. Thus, the authors clearly demonstrated 295 that the O<sub>2</sub>-absorbing laminate removed oxygen before it was otherwise available for 296 297 degrading reactions in the food product.

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A novel iron-based oxygen scavenger using iron nanoparticles, blended with activated 299 carbon, sodium chloride, and calcium chloride, was produced and evaluated by Mu and 300 301 others (2013). Nano-sized iron-based oxygen scavenger (110 nm average particle size) 302 exhibited a higher oxygen-scavenging rate (estimated 13.5 mL/d) compared to the oxygen scavenger-containing microscale pure iron powder (about 20  $\mu$ m; estimated 1.8 mL/d). 303 304 Moreover, the scavenger capacity of the nano-sized scavenger was found to be almost 1.4 305 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 306 and walnuts over a period of 120 days showed that the iron nanoparticles were an effective 307 means of inhibiting lipid oxidation. While the peroxide values (PV) of the control samples 308

309 without OS increased (sunflower seeds: from 4.32 to 46.89 meq O<sub>2</sub>/kg oil, walnuts: from 2.41 to 21.85 meq O<sub>2</sub>/kg), the PV of the samples containing the nano-sized oxygen scavenger 310 311 were shown to be significantly lower (sunflower seeds:  $19.82 \text{ meg } O_2/\text{kg}$ , walnuts: 8.84meq O<sub>2</sub>/kg) after 120 days of storage. Similar results were obtained for secondary lipid 312 oxidation. Although significant differences in the *p*-anisidine values (AnV) between the 313 samples were only observed after 40 and 80 days of storage of sunflower seed and walnut 314 315 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 316 317 reduce consumer acceptance due to possible leakage and unintentional consumption.

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319 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 320 defects, particularly when these defects have a critical size that is below the detection limit 321 of standard leak testers of 10 µm (Sasaki and Kamimura 1997; Sängerlaub and others 322 2013a). In this context, Sängerlaub and others (2013a) simulated food packages with pinhole 323 324 defect sizes of 10  $\mu$ m. They performed long-term storage experiments (300 days) to 325 compare O<sub>2</sub> 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 326 oxygen could not be fully prevented, oxidation reactions were significantly reduced by the 327 application of the OS film. Salami samples packed with OS showed less difference in color 328 ( $\Delta E$  of 5.4) compared to those packed without OS ( $\Delta E$  of 6.9). Additionally, the use of OS film 329 330 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 331

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

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Ascorbic Acid. Matche and others (2011) modified LLDPE films by blending them with iron 335 and ascorbic acid (Fe/AA) or zinc and ascorbic acid (Zn/AA). Thereby, the ascorbic acid (AA) 336 337 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 338 an OS performance of 47.6 and 37.4 mL, respectively, in 750 hours. With the application of 339 the OS films in the form of sealed bags to bakery products, an overall shelf-life extension of 340 341 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 342 343 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, 344 respectively, after 4 days. This was explained by the lower water vapor transmission rates 345 (WVTR) of both Fe/AA (17.2) as well as Zn/AA films (17.4) compared to the pure LLDPE film 346 347  $(20 \text{ g/m}^2 100 \text{ gauge/day})$ , which was used as a control. Moreover, both modified variations 348 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 349 acceptable (softness and taste) up to 5 and 6 days, respectively, whereas the control 350 351 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 352 353 headspace oxygen concentration of the packaged bakery products. This hinders the

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

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Photosensitive dyes. Maloba and others (1996) used a photosensitive OS film to improve 357 oxidative stability of sunflower oil. The applied ethyl cellulose polymer film contained the 358 common organic dyes eosin and curcumin and the synthesized polyether polyfuryloxirane 359 (PFO). When exposed to light, this OS film uses energy transfer to convert triplet oxygen 360  $({}^{3}O_{2})$  into highly reactive singlet oxygen  $({}^{1}O_{2})$ , which is absorbed irreversibly by the PFO. The 361 method and mechanism behind such systems are explained by Rooney (1995). The 362 sunflower oil was stored in the presence of the OS film and oxidative stability was evaluated 363 by determination of peroxide values (PV) and gas-chromatographic (GC) measurement of 364 headspace hexanal. To evaluate the influence of illumination, an initial irradiation period of 2 365 days at 2000 lux was followed by continuous illumination at normal room light (500 lux), the 366 latter imitating the light level commonly encountered on retailer's shelves. Control samples 367 were additionally stored under light exclusion. It was shown that sunflower oil stored under 368 369 illumination, at 23 °C and 12 weeks of storage, in the presence of the OS film exhibited 370 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 371 372 film, dark), about 75 meq/kg (no OS film, illuminated + antioxidant) and about 100 meq/kg 373 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 374 375 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 376

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.

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A similar OS film has also been successfully implemented with orange juice to protect juice 382 383 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 384 triggered by UV illumination just prior to packaging. The authors demonstrated that at 25 385 and 4 °C, the initial dissolved oxygen concentration of 2.7 ppm in the orange juice samples 386 packed in OS pouches was reduced to below 0.04 ppm within 3 and 7 days, respectively. In 387 contrast, for the control pouches without OS, dissolved oxygen in the orange juice was 388 above 0.04 ppm up to at least 35 and 77 days, respectively, clearly reflecting the impact of 389 the competition for the oxygen between the OS film and the ascorbic acid as well as the 390 impact of temperature. This was confirmed by the correlating ascorbic acid retention which 391 392 was significantly higher for the samples using the OS pouches with 30.0% (25 °C) and 73.2% 393 (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 394 395 ascorbic acid also correlated with an increase in the non-enzymatic browning of the juice. 396 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 397 398 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 399

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.

405

In dairy products such as probiotic yogurt, the application of oxygen scavengers can be of 406 particular interest since dissolved oxygen has a negative effect on the viability of probiotic 407 bacteria such as Lactobacillus acidophilus and Bifidobacterium spp. which are essential for 408 409 yogurt production (Shah and others 1995). To control the amount of dissolved oxygen, Miller 410 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), 411 412 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 413 methods of probiotic yogurt as well as different packaging systems, and they evaluated their 414 415 effect on the dissolved oxygen content during a normal shelf-life for yogurt (42 days). Best 416 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 417 418 3 ppm (normal container) and 1.7 ppm (OS-container) after the first day. It further 419 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 420 421 regarding the probiotic bacteria which require low oxygen concentration for postfermentation, thereby leading to a product with increased health benefit. In practical 422

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

425

In ultra-high temperature (UHT) milk, oxygen scavengers have been shown to prevent the 426 development of stale flavor. Perkins and others (2007) packaged indirectly processed UHT 427 milk using packaging films containing a prototype ZerO<sub>2</sub><sup>TM</sup> OS film laminate. Thereby, the OS 428 film significantly reduced the initial dissolved oxygen content of ~7 mg/L to ~3.8 mg/L during 429 14 weeks of room temperature storage (26 °C), compared to the control with ~5 mg/L. 430 Significant reductions of 23-41% were also observed for stale flavor volatiles such as methyl 431 ketones and aldehydes. Regarding lipid oxidation, a gradual increase in total free fatty acid 432 levels was observed during the 14-week storage period of samples without OS (data of OS 433 not published). However, the levels remained far below threshold values, indicating low 434 lipolytic rancidity. As a consequence, the consumer panel failed to detect a significant 435 difference in odor between the samples with and without OS. The authors concluded that 436 sensory analysis would have better reflected the rancid off-flavor, however, a lack in 437 438 regulatory approval for the OS prototype used in their study precluded taste-testing. 439

Unsaturated hydrocarbon dienes. Baiano and others (2004) incorporated an oxygenscavenging 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 446 authors demonstrated that the use of OS bottles could significantly slow down the 447 448 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 449 even 3-4 times lower at the usual refrigerated storage of 5 °C. The authors reasoned the 450 451 inferior results with PET and glass were due to the oxygen permeability of PET and the 452 presence of pro-oxidant substances and catalysts in glass containers. The results demonstrated that glass containers could be advantageously replaced with polymeric 453 bottles including an oxygen scavenger, particularly in the case of beverages containing 454 ascorbic acid. However, the application of a real fruit juice instead of a model system would 455 have been preferable. Using the above-mentioned OS polymer in the form of a cast-456 extruded monolayer-PET film, Galdi and Incarnato (2011) demonstrated the prevention of 457 the browning of bananas. Fresh-cut banana slices were shown to have significantly less 458 (~50%) color difference ( $\Delta E$ ) after three days wrapped in the OS film compared with the 459 conventional PET film. Later optimizations of these OS PET films resulted in co-extruded 460 multilayer films where the internal active layer was protected from fast oxidation by 2 461 462 external layers of pure PET (PET/OS-PET/PET) in order to increase the reaction time (Di Maio and others 2015). 463

464

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 469 product/headspace ratio (Andersen and Rasmussen 1992; Møller and others 2000; 470 471 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 472 initial headspace oxygen (Matche and others 2011). Recently, a rapid OS system based on a 473 catalytic system with palladium (CSP) has been developed. Palladium was coated on 474 475 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 476 oxygen in food packages if hydrogen was included in the modified atmosphere (MA) of the 477 packaging (Yildirim and others 2015). Due to its high efficiency, this film is particularly 478 suitable for food products which are very susceptible to oxygen and where the oxidation 479 480 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 481 2 vol.-% initial oxygen in the headspace (160 cm<sup>3</sup>) of MA-packed ham within 35 min after 482 packaging. In this way, the color of the ham could be preserved and discoloration prevented 483 484 for 21 days of storage, although packages were exposed to light 24 h/day. In contrast, 485 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, 486 487 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 488 slices, and gluten-free bread slices. At normal and modified atmosphere without OS film, 489 490 visible mold growth was detected in all samples after 2 days with a simultaneous decrease in

headspace oxygen concentration. In contrast, in MA packages with OS film, mold growth was
retarded up to 8-10 days, resulting in a 3-4 fold longer shelf-life for all types of bread tested.

Apart from its high efficiency, the applied CSP also has some limitations. First of all, 494 hydrogen is required, therefore, the application of the CSP is limited to products packed 495 496 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 2.5 vol.-% (Yildirim and others 2015). Another drawback is that the catalytic activity of the 498 palladium-based system may be inhibited or even inactivated by volatile sulfur compounds 499 present in the headspace of certain packaged food products (Röcker and others 2017). With 500 501 regard to the safety of palladium, the EFSA published a scientific opinion on the safety assessment of the palladium metal and hydrogen gas for use in active food contact 502 materials. The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing 503 Aids (CEF) concluded that "the active substances palladium and hydrogen do not raise a 504 safety concern for the consumer when used as an oxygen scavenger in packages for foods 505 506 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 prevents the migration at detectable levels" (EFSA CEF Panel 2014). 508

509

Implementation of OS technologies into food packaging operations is often challenging as OS systems are sensitive to environmental conditions, such as temperature and pH (Galdi and others 2008; Solovyov 2010; Damaj and others 2014), or require humidity (Solovyov 2010) or 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.

521

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.

529

#### 530 Moisture Scavengers

531 Moisture content and water activity are critical factors affecting the quality and safety of 532 various types of foods (Labuza and Hyman 1998). For instance, many dry products are 533 sensitive to humidity during storage, and even low relative humidity (RH) levels inside the 534 packages may cause significant quality deterioration. Increase in moisture makes the 535 products more prone to microbial spoilage and may cause alterations in texture and 536 appearance, consequently reducing shelf-life (Labuza and Hyman 1998, Day 2008). For other 537 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).

542

Moisture control strategies in packaging can be divided into categories, such as moisture 543 544 reduction (for example, by MAP through replacing the humid air in the headspace with dry 545 modified atmosphere gas, or vacuum-packaging through the removal of humid air in the headspace), moisture prevention (by barrier packaging), and moisture elimination (by 546 547 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 548 549 those that lower the humidity without any active ingredients, such as micro-perforated films (for example, Xtend<sup>®</sup> films, Israel) (Suppakul 2015), and other packaging materials that are 550 inherently hygroscopic, such as those that are 100% cellulose-based (as defined in (EC) No 551 450/2009 (European Commission 2009)). Humidity levels inside packages can also be 552 553 controlled through the appropriate selection of packaging materials with a high barrier 554 against water vapor.

555

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

561 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 562 563 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 564 PS sheets, or cellulose, combined with superabsorbent polymers/minerals/salts 565 (polyacrylate salts, carboxymethyl cellulose, starch copolymers, silica/silicates) (Ščetar and 566 others 2010). Moisture absorbing pads are not often considered to be active packaging. 567 According to the EU Guidance to the Commission Regulation (EC) No 450/2009, "Materials 568 and articles functioning on the basis of the natural constituents only, such as pads composed 569 of 100% cellulose, do not fall under the definition of active materials because they are not 570 571 designed to deliberately incorporate components that would release or absorb substance." However, moisture absorbing pads containing components that "are intentionally designed 572 to absorb moisture from the food" and can be considered as active packaging (European 573 Commission 2009). Absorbing pads can also be used in combination with antimicrobials (for 574 example, Dri-Fresh<sup>®</sup>Fresh-Hold<sup>™</sup> ABM, Sirane, USA), pH control agents and/or carbon 575 dioxide generators/oxygen scavengers, to avoid certain shortcomings, such as odor 576 577 generation or leakage.

578

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 depends on its water vapor sorption isotherm (Sängerlaub and others 2013b). Desiccants are
commonly placed into packages in the form of sachets, micro-porous bags, or are integrated
in pads. Some examples applied to food products are given in Table 3, however, pads are
excluded as they are already successfully applied on the market.

588

Zeolites. Zeolites have a significant tendency to attract moisture and can also release the absorbed water without any change in the crystalline structure and moisture absorption properties (Julkapli and Bagheri 2016). By virtue of these properties, natural nanozeolite can be added to pulp/paper and plastic films to regulate the amount of moisture absorbed in packaging (Julkapli and Bagheri 2016), however, its application is still limited to non-food packaging (Wu and others 2010).

595

Bentonite/sorbitol/calcium chloride. One of the potential application of moisture 596 scavengers is packaging of mushrooms since the quality of the mushrooms is strongly 597 influence by the RH in the headspace. Humidity below 86% RH promotes moisture and 598 599 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 optimal humidity in the headspace of packaging for mushrooms was found to be 96% 601 602 (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 and short shelf-life (1-3 days at ambient temperature). In this study, combinations of fast-604 605 absorbing (CaCl<sub>2</sub>, KCl, and sorbitol, with moisture holding capacity  $0.91 \pm 0.01$  [g H<sub>2</sub>O/g] in 120 h) and slow-absorbing moisture absorbers (bentonite/sorbitol, with moisture-holding 606

607 capacity 0.34 ±0.02 [g H<sub>2</sub>O/g] in 120 h), were tested (Table 3). The best combination of absorbers was found to be bentonite/sorbitol/CaCl<sub>2</sub> in proportions of 0.55:0.25:0.2 g/g 608 609 desiccant, respectively. The study revealed that the moisture holding capacity of the scavenger is dependent on the relative humidity; it increased from 0.51 to 0.94 g water per g 610 desiccant when the relative humidity was increased from 76% to 96%. A change in 611 temperature (from 4 to 16 °C) did not have a significant influence on the moisture-holding 612 613 capacity. A positive impact of desiccant usage was observed, namely a decrease in the moisture condensation inside the packaging, and improved transparency of the packaging 614 film. Mushrooms (250 g) packed with 5 g of bentotite/sorbitol/CaCl<sub>2</sub> desiccants resulted in a 615 lower browning index (BI 14.8) compared to those packed without desiccants (BI 18) after 5 616 days of storage at 10°C. However, for packages with higher levels of desiccants (10 or 15 g), 617 618 browning indices were greater due to the browning and excessive moisture loss. The appearance of mushrooms packed with 5 g desiccants was also better since higher amounts 619 resulted in excessive moisture loss. After 5 days, the mushrooms packed with 5 g of 620 desiccants were still sellable on the market. The results show that the capacity of the 621 622 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 experiments were performed by Azevedo and others (2011). A mixture of desiccants 624 consisting of calcium oxide/calcium chloride/sorbitol in a ratio of 0.5:0.26:0.24, respectively, 625 resulted in a moisture-holding capacity of 0.81 g water per g of desiccant. However, no food 626 application was evaluated in this study. 627

628

629 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 630 631 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 632 integrated into sealed containers (material not stated) containing fresh maize with a water 633 634 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 635 with SAP-to-maize-ratios of 1:5 and 1:1 resulting in aflatoxin contents of 33 or <3 ng/g, 636 respectively. Aflatoxin contamination could be reduced to <4 ng/g, even at 20, 30 and 40 °C 637 drying temperatures, with a 24 h frequency change of the SAP (1:5), complying with current 638 Kenyan and European legislation that limit aflatoxin content to 10 and 4 ng/g, respectively. 639 The application of poly(acrylic acid) sodium salt therefore shows potential for grain drying in 640 641 reducing aflatoxin contamination, particularly in developing countries as it is a cheap and reusable solution. 642

643

644 Sodium chloride and hygroscopic ionomer. Langowski and others (2006) and Sängerlaub 645 and others (2013b) developed salt-embedded, humidity-regulating trays consisting of a 3layer structure: barrier layer, active layer with NaCl and sealing layer, to control the humidity 646 647 in food packages. Thereby, the active layer was foamed and stretched to form cavities 648 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 649 650 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 651

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

671

Tamarind seed galactoxyloglucan. Polysaccharides, such as galactoxyloglucan from tamarind
seeds, can be used as moisture-absorbing aerogels. Such aerogel-based packaging systems,
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.

680

Commercial moisture absorbers can be found in a variety of formats including as absorbing
pads, such as Cryovac<sup>®</sup>Dri-Loc<sup>®</sup> (Sealed Air Corporation, USA), Thermasorb (Thermasorb PVT
Ltd., Australia), and MeatGuard<sup>®</sup> or MeatPad<sup>®</sup> (McAirlaid's Inc., USA); absorbing films, such
as Pichitto/Pichit (MTC Kitchen, Japan), MoistCatch<sup>™</sup> (Kyodo Printing Co., Ltd., Japan), and
Active Film<sup>™</sup> (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<sup>®</sup> (Maxwell Chase Technologies, LCC, USA).

688

As described in this section, various moisture scavenging systems can be applied to preserve 689 690 quality and prolong the shelf life of food. The examples presented above underline the 691 importance of product-adjusted moisture scavenging systems for food applications. The most well-established and commercially well-recognized technologies incorporate the use of 692 693 sachets, pouches (including desiccants) or pads – devices that do not interfere with the 694 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 695 696 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). 697

698

### 699 Ethylene Absorbers

700 Ethylene ( $C_2H_4$ ) is a growth-stimulating hormone (plant growth regulator) accelerating 701 ripening and senescence through increasing the respiration rate of fresh and minimally processed climacteric produce and shortening the shelf-life during postharvest storage. 702 703 Ethylene also accelerates chlorophyll degradation rates, especially in leafy products, and enhances excessive softening of fruits (Saltveit 1999; Ozdemir and Floros 2004). For these 704 705 reasons, the removal of ethylene from the product environment by application of ethylene 706 scavengers slows ripening and senescence, thereby enhancing quality and prolonging shelf-707 life.

708

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<sub>4</sub>) in order to oxidize/inactivate ethylene (Floros and others 1997; Ayhan 2011; Llorens and others 2012) However, KMnO<sub>4</sub> is never used in direct food contact due to its high toxicity (Martínez-Romero and others 2007).

716

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

permeability of the film so that carbon dioxide can diffuse faster and oxygen can enter more
readily than through pure polyethylene to obtain an equilibrium atmosphere (De Kruijf and
others 2002; Esturk and others 2014).

724

Metals and metal oxides are also good candidates for ethylene removal. Photoactive TiO<sub>2</sub> is 725 726 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 should be considered. Nano-silver is also claimed as an ethylene blocker; however, it has 728 been tested in absorbent pads which were placed in trays of fresh-cut melon and not in the 729 packaging structure (Hu and Fu 2003; Fernández and others 2010). Palladium-based 730 731 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 2015) or in a storage room (Martínez-Romero and others 2009), not in the structure of 733 packaging films. The high cost of palladium has been assumed to limit its industrial 734 application (Martínez-Romero and others 2007). Abe and Watada (1991) reported that 735 736 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 softening in fresh-cut kiwifruit and bananas, and chlorophyll loss in spinach leaves, but not 738 739 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

metal trays with a glass cover, however, it was acknowledged that this type of high barrier
packaging is not suitable for products that respire.

745

Sothornvit and Sampoompuang (2012) incorporated activated carbon (30%) with 0.3% of the 746 polysaccharide glucomannan into paper made of rice straw. The active material adsorbed 747 748 0.69 µL/L ethylene with a scavenging capacity of 77%. The ethylene adsorption capacity per 749 surface area was calculated as 34.2  $\mu$ L/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 permanganate) designed as an active packaging for fresh fruits and vegetables. However, in 754 755 this study, there was no application to prove the effect in a real food system (Taboada-756 Rodríguez and others 2013).

757

The incorporation of scavengers in packaging films may be a better option to solve sachet-758 related problems. Ethylene scavengers could either be embedded into a solid, dispersed in 759 plastic, or incorporated into various layers of the packaging (Ozdemir and Floros 2004). 760 761 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 ethylene scavengers incorporated into the actual packaging material, rather than in sachet 763 format, for fresh produce. Ethylene scavenger can prolong the shelf life of climacteric fruits, 764 765 such as apples, kiwifruit, apricot, bananas, mango, cucumber, tomato, and avocados, and

vegetables, such as carrots, potatoes, and asparagus (De Kruijf and others 2002). The list of
 different produce packaged with different packaging film and ethylene absorber and the
 benefits of such systems are presented in Table 4.

769

**Nano-particles.** Nano-TiO<sub>2</sub> is reported to oxidize ethylene into  $H_2O$  and  $CO_2$  (Han and Nie 770 771 2004). Yang and others (2010) tested PE blended with nano powders of Ag, TiO<sub>2</sub>, and kaolin for preservation of fresh strawberries at 4 °C for 12 days. Results showed that active PE with 772 773 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 66.3 μmol/g for nano-packaging and 75.4 μmol/g for normal packaging), and enzyme activity 777 of polyphenol oxidase and pyrogallol peroxidase. However, the gas composition including 778 779 ethylene in the headspace was not monitored in this study and there is no indication of shelf life. 780

781

Chinese bayberries were packaged by Wang and others (2010) with active PE including 30% nano powder of Ag, TiO<sub>2</sub>, and kaolin-clay or treated with hot air or the combination of hot air treatment and nano-packaging, and stored at 1 °C and 80-90% RH for 8 days. Results showed that the application of hot air (48 °C for 3 h) and/or active packaging reduced the incidence of green mold decay (from 75.5% to 34.6% for active packaging and to 22.7% for hot air treatment and to 14.8% for the combined treatment), fruit respiration, and ethylene 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.

794

Li and others (2009) studied the effect of active packaging produced by blending nano 795 powders of nano-Ag, nano-TiO<sub>2</sub>, and kaolin with polyethylene for the preservation of 796 797 Chinese jujube. The active packaging improved the physicochemical and sensory quality of 798 the product compared to polyethylene without nano-powder (control). Application of active 799 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 800 801 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 802 reported as 17.6  $\mu$ L/kg h for the control on the third day and 9.2  $\mu$ L/ kg h for nano-packaging 803 on the sixth day of storage. The active packaging is recommended for Chinese jujube to 804 805 improve quality, however, a specific shelf life was not indicated.

806

Hu and others (2011) studied the effect of PE blended with nano-Ag, nano-TiO<sub>2</sub>, 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 812 813 researchers stated that nanocomposite packaging was effective in inhibiting ethylene production (57.4% of lower headspace ethylene in active packaging), preventing 814 physiological changes, and delaying ripening, however, no specific shelf life was indicated. A 815 lower level of ethylene production was related to the synergistic effect of nanoparticles 816 817 which decompose or oxidize ethylene into water and carbon dioxide (Li and others 2009). 818 Li and others (2011) tested poly(vinyl chloride) (PVC) film coated with nano-ZnO powder on 819 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 820 821 compared to uncoated PVC (control). Maximum ethylene content was reported as 40  $\mu$ L/kg day for nano-packaging on the ninth day and, 70 µL/kg day for the control on the sixth day of 822 storage. The browning index was significantly reduced from 31.7 to 23.9 on day 12, 823 maintaining the initial appearance. The activity of polyphenol oxidase was 9.6 U/g min in 824 825 active packaging and 21.5 U/g min in normal packaging on day 9. The authors reported that 826 the nano-packages had more oxygen and less carbon dioxide in the headspace compared to 827 the control, indicating the lower respiration in the nano-coated PVC. Nano ZnO is reported to have similar physical properties to TiO<sub>2</sub> which oxidizes ethylene into water and carbon 828 829 dioxide under UV irradiation (Han and Nie 2004).

830

831 Zeolite-based minerals. Zeolite-based ethylene absorbers are good candidates for

832 commercial use. The most characteristic property of zeolites is their porous three-

dimensional structure with cation exchange, adsorption, and molecular sieving properties.

834 Therefore, zeolites have been used in many industrial and agricultural applications, including

as an ethylene-absorbing additive incorporated into packaging films. There are various 835 reports that incorporation of zeolites increases gas permeability of packaging films by means 836 of their crystalline porous-three-dimensional framework structure (Süer and others 1994; 837 Kittur and others 2005; Zhao and others 2011). Esturk and others (2014) successfully applied 838 low-density polyethylene (LDPE) bags with ethylene absorber (8% Tazetut<sup>®</sup> master batch, an 839 840 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 841 stated that spoilage occurred quickly in unpackaged broccoli (control) illustrated by 842 chlorophyll degradation, stem-hardening, and mass loss of 41.5% on day 20, which was less 843 than 1% for packaged applications. The product (control) was unacceptable for the sensory 844 845 panel after 5 days. However, the quality loss was significantly reduced in active LDPE bags 846 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 847 848 films extended the shelf life of broccoli up to 20 days, compared to a 5-day shelf-life for the unpackaged product. 849

850

The quality of kiwifruit packaged with HDPE bags including a sachet with KMnO<sub>4</sub> impregnated zeolites at 4 °C for 31 days was reported by Küçük (2006). 0.2 mL KMnO<sub>4</sub>/g zeolite was impregnated with zeolites of 1-3 mm in size. KMnO<sub>4</sub> 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.

862

863 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-864 permeable film with ethylene-absorbing property, micro-perforated highly gas-permeable 865 film and common low-density polyethylene film. The non-perforated film with ethylene 866 absorber extended the shelf life of mango to 40 days at 12 °C, compared to 35 days with the 867 868 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 869 870 weight loss, maintained firmness, and there was no sign of decay during storage. Low 871 ethylene concentrations (<3.5  $\mu$ L/L) were reported in mangoes with the different packaging films. Ethylene production in the microperforated packages was higher than that in the non-872 perforated and non-perforated with ethylene absorber packaging. The ethylene-absorbing 873 characteristics of the material delayed the ripening process of mangoes. A further study 874 shows that zeolite-added LDPE bags were applied to ripe kiwifruits successfully establishing 875 876 an equilibrium atmosphere in the headspace in 5 days, however, the control material with 877 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 878 879 kiwifruits using zeolite-incorporated LDPE bags. Ethylene measurement is not reported in this study (Ayhan 2016). 880

Jacobsson and others (2004) tested 4 different materials on fresh broccoli at 2 storage 882 temperatures (4 and 10 °C). Among the materials tested, one material was LDPE-based with 883 pouches containing a commercial sachet (Ryan Instruments, The Netherlands) to absorb 884 ethylene, and the other material was commercial LDPE film impregnated with a natural 885 hydroscopic mineral produced by PEAKfresh® (USA). Results showed that LDPE pouches with 886 887 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 888 889 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 890 891 loss in weight, better color and texture, while the chlorophyll content was maintained. This publication does not report any ethylene measurements. 892

893

894 Commercially-available ethylene-scavenging films in the market are mostly zeolite-based such as Evert-Fresh® (Evert-Fresh Corporation, USA), PEAKfresh® (PEAKfresh®, USA), 895 Profresh<sup>®</sup> (E-I-A Warenhandels GmbH, Austria) and Bio-Fresh<sup>™</sup> (Grofit Plastics, Israel). The 896 main limitation of these films is opacity and, thus, these plastics are mostly colored 897 (Martínez-Romero and others 2007; Ayhan 2013). PEAKfresh<sup>®</sup> is a polyethylene bag 898 899 impregnated with minerals to absorb ethylene and moisture. Evert-Fresh<sup>®</sup> absorbs ethylene, 900 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 901 Profresh<sup>®</sup>. Bio-Fresh<sup>™</sup> is a film used in combination with modified atmosphere packaging to 902 903 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).

907

Packaging materials integrated with ethylene-removers in the packaging structure are still 908 limited in commercial applications. The use of these materials for a broad spectrum of fresh 909 910 products is also limited compared with other active packaging applications reported in the 911 literature. The main principle for the successful packaging of fresh and fresh-cut produce is 912 that the gas permeability (oxygen and carbon dioxide) of the packaging film and respiration 913 rate of the produce should correspond allowing gas equilibrium in the headspace, as well as 914 removal of ethylene from the package environment (Ayhan 2013). The application of adequate oxygen and low carbon dioxide-modified atmosphere packaging combined with 915 916 ethylene absorber could provide further benefits to control the product metabolism and 917 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-918 919 specific, since each produce varies in respiration rate, ethylene production rate, and 920 ethylene sensitivity, and hence the requirements for packaging and storage vary.

921

### 922 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.

931

Torres-Arreola and others (2007) reported a delay in lipid oxidation and protein 932 933 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 934 oxidation, expressed as thiobarbituric acid-reactive substances (TBARS) values  $(4.20 \pm 0.52)$ 935 vs 11.95 ±1.06 mg malonaldehyde (MDA)/kg), peroxide index values (7.20 ±1.38 vs 15.15 936 937 ±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 938 939 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 940 use of natural antioxidants such as  $\alpha$ -tocopherol. Poly(lactide-co-glycolide) films loaded with 941 942 2% α-tocopherol, or a combination of 1% BHT and 1% BHA, were used to evaluate the 943 stability of dry whole milk and dry buttermilk (Van Aardt and others 2007). BHT and BHA have a higher volatility than  $\alpha$ -tocopherol, therefore it was expected that they would be 944 945 more suitable for dry food applications. However, in this study,  $\alpha$ -tocopherol offered the same antioxidant protection for whole milk powder exposed to light and oxygen. In a further 946 study, sealable multilayer films (HDPE/EVOH/LDPE) manufactured with an inner LDPE layer 947 containing 4% of  $\alpha$ -tocopherol delayed the lipid oxidation of whole milk powder at 30 and 40 948 °C (Granda-Restrepo and others 2009b). Similarly, sealable LDPE films containing 1.9 and 3% 949

of  $\alpha$ -tocopherol maintained the oxidation stability (hexanal content) of corn oil for 16 weeks 950 at 30 °C, compared to 12 weeks for the oil in a control bag without antioxidant (Graciano-951 Verdugo and others 2010). Manzanarez-López and others (2011) reported that poly (lactic 952 acid) films containing 2.58% of  $\alpha$ -tocopherol were also able to delay the induction of the 953 oxidation, measured as peroxide value, of soybean oil at 20°C (max. values of 9.9 vs 19.5 954 meq/kg), 30°C (max. values <10 vs 27.5 meq/kg), and 40°C (max. values of 13.5 vs 33.9 955 956 meq/kg). Meanwhile, Torrieri and others (2011) observed that with the combined use of 957 MAP and LDPE-embedded  $\alpha$ -tocopherol packaging, lipid and fat oxidation in fresh bluefin tuna fillets could be reduced. Several natural antioxidant products (TOCOBIOL®-PV, 958 NUTRABIOL®-T90, NUTRABIOL®-T50 PV) containing tocopherols incorporated into LDPE films 959 960 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). 961

962

Active antioxidant food packaging films produced by incorporating 4.6% of the natural 963 flavonoid quercetin into EVOH matrix showed enhanced lipid oxidative stability, as 964 demonstrated by a lower peroxide index (12 vs 27 meq/kg) and a reduction in TBARS values 965 by 25% during storage time (López-de-Dicastillo and others 2012b). Catechin, an active 966 component of green tea with properties similar to those of quercetin, was shown to be an 967 968 effective antioxidant ingredient to retard the oxidation of sunflower oil and fried peanuts 969 (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 °C for 40 days resulted in a 970 strong reduction in hexanal content released into the headspace up to 25 days, after which 971 972 the hexanal increased at the same rate as in the control sample. Additionally, the peroxide

973 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 974 975 films actively protected the oil. Moreover, the films with guercetin (0.76 and 4.01%) were more effective compared to those with catechin due to the higher solubility of quercetin in 976 this product, as well as its higher antioxidant capacity. The reported results using accelerated 977 shelf life testing (37 °C) to estimate the antioxidant potential of the films. However, the 978 979 antioxidant performance under real storage conditions needs to be evaluated before commercial implementation. Another issue to be addressed would be the inclusion of EVOH 980 in a multilayer system in order to protect the system from water and to reduce costs, while 981 982 maintaining antioxidant activity. Other nonvolatile antioxidants such as ascorbic, ferulic, and 983 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 984 ground beef (López-de-Dicastillo and others 2012b; Júnior and others 2015). 985

986

Antioxidant packaging systems containing volatile extracts, essential oils, or active 987 988 components of plants or spices have been developed to improve quality and to extend the 989 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 990 991 effectively inhibited lipid oxidation and had a positive effect on the stability of beef patties 992 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 993 994 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). 995

996

997	Antioxidant films obtained from biomaterials containing green tea extract have been
998	demonstrated to improve oxidative stability of pork meat products (Siripatrawan and Noipha
999	2012; Yang and others 2016). Yang and others (2016) reported a stronger antioxidant
1000	capacity of films obtained with 0.5% of green tea extract compared to those obtained with
1001	oolong and black tea extracts. Specifically, at the end of 10 days storage of pork meat, the
1002	TBARS value of the control sample was 1.64 mg MDA/kg, whereas the TBARS values of the
1003	samples wrapped with film containing green tea extract, oolong tea extract, and black tea
1004	extract were 0.93, 1.16, and 1.27 mg MDA/kg sample, respectively. In another study,
1005	Lorenzo and others (2014) reported that multilayer barrier films containing oregano
1006	essential oil (2%) were more effective in preventing lipid oxidation of foal meat packed in
1007	MAP (80:20, $O_2$ :CO <sub>2</sub> ) than those with green tea extract (1%). An interesting investigation by
1008	Carrizo and others (2016) reported radical-scavenging capacity of sealable multilayer films,
1009	in which green tea extract was added to the laminating adhesive and thus not in direct
1010	contact with the packaged food (peanuts and cereals covered with chocolate). According to
1011	the authors, this packaging system was able to protect food against oxidation during a long-
1012	term period of 16 months. It is important to highlight the industrial relevance of this study,
1013	since the use of commercial packaging materials would facilitate industrial implementation
1014	of this technology.
1015	
1016	Other authors have explored the application of packaging materials containing rosemary and

1017 oregano extracts in direct contact with muscle foods as active packaging systems.

1018 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). 1019 1020 Oregano extracts included in the films were more efficient in preventing oxidation of lamb 1021 meat than rosemary extracts, they extended fresh odor and color from 8 to 13 days 1022 compared to the control (Camo and others 2008). Some authors have studied the impact of 1023 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 1024 protect meat patties from high-pressure-processing-induced lipid oxidation and 1025 1026 consequently extend the shelf life. More specifically, the lipid oxidation of chicken breast 1027 patties submitted to high-pressure treatment and stored at 5 °C was higher in the surface 1028 part of samples and the active packaging delayed oxidation it up to 25 days demonstrated by 1029 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 1030 acid), and TBARS (4.20±0.52 vs 11.95±1.06 mg MDA/kg) (Bolumar and others 2011).

1031

1032 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 1033 1034 beef with LDPE film coated with a brewery residual waste extract was able to reduce lipid 1035 oxidation by up to 80% during cold storage (Barbosa-Pereira and others 2014). Barley husk, 1036 another waste product obtained from the brewery industry, also proved to be effective in 1037 slowing down lipid hydrolysis and improving the oxidative stability in blue shark muscle 1038 (Pereira de Abreu and others 2011). Meanwhile, anthocyanins from wine grape pomace, beet root residue powder, and mango and acerola pulp incorporated into sealable 1039 biodegradable films had a protective effect on sunflower and palm oil oxidation (Souza and 1040 1041 others 2011; Oliveira and others 2016; Stoll and others 2016). For example, a sunflower oil

1042 control sample directly exposed to the air and light reached a peroxide index of 65.8 meq/kg 1043 after 3 days, while the samples stored in cassava starch film bags prepared with encapsulated anthocyanins presented lower values (4.7-28.7 meg/kg) (Stoll and others 1044 1045 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 1046 high concentrations of mango and acerola pulp additives (Souza and others 2011). However, 1047 it was found that vitamin C in acerola pulp acted as a pro-oxidant agent, which suggests that 1048 1049 the use of components rich in vitamin C should be avoided.

1050

1051 Extensive research on the use of antioxidant packaging systems to prevent food oxidation 1052 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 1053 1054 their target market and consequent legal status. Therefore, to favor the industrial 1055 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 1056 1057 film processing techniques (such as extrusion or coating vs solvent-casting), packaging 1058 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 1059 1060 properties of food, and validation using real storage conditions.

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# 1062 Carbon Dioxide Emitters

The antimicrobial effect of CO<sub>2</sub> is thoroughly documented in the literature (Kolbe 1882;
Valley 1928; Haas and others 1989; Debs-Louka and others 1999). CO<sub>2</sub> is soluble in the

aqueous and fatty phases of food products and the antimicrobial effect is highly dependent 1065 on the rate of solubility and amount of CO<sub>2</sub> dissolved in the food product. The solubility of 1066 carbon dioxide increases with decreasing temperature (Devlieghere and others 1998; 1067 Devlieghere and Debevere 2000) and also varies for different food products depending on 1068 the properties of the food such as surface area, pH and composition (water, fat, protein) 1069 (Chaix and others 2015). The antimicrobial effect has been found to be proportional to the 1070 partial pressure of the gas (Blickstad and others 1981). In terms of food packaging, this 1071 1072 implies that the total amount of  $CO_2$  present in the headspace of the package is crucial for 1073 the effect. The concept of a CO<sub>2</sub>-releasing device to be implemented in modified 1074 atmosphere packages (MAP) to maintain high headspace levels of CO<sub>2</sub> during storage, and 1075 thereby, facilitate smaller package volumes (lower gas to product (g/p) volume ratio), and prolonged shelf life was introduced in the 1990s. 1076

1077

1078 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 1079 1080 impact. The release of carbon dioxide from a tuned emitter system may also prevent 1081 packaging deformation as it compensates for CO<sub>2</sub> absorption into the food product in the initial stages of storage. In this way it counteracts formation of negative pressure in MA-1082 1083 packages that increase the drip loss of the product, which may give the packages an 1084 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 1085 CO<sub>2</sub> levels in the packages will have a knock-on effect in the form of a reduction in food 1086 1087 waste, an issue gaining increasing attention and priority in western parts of the world.

1088

1089	The emitters usually come in the form of a pad or sachet, in many cases as a combined liquid
1090	absorber. The active ingredients inside the absorbent pad react when the pad absorbs liquid
1091	that is seeping out of the product, resulting in the release of $CO_2$ . Over the last decade, the
1092	field of $CO_2$ emitters has advanced significantly, reflected in increased research activity and
1093	sale of commercial $CO_2$ emitters. Table 6 lists the $CO_2$ releaser technologies available for
1094	food preservation to date, as well as their applications and benefits to specific food
1095	products. In the literature, there are several reports of the use of ferrous carbonate in
1096	carbon dioxide emitters (Rooney 1995; Sivertsvik 2003; Restuccia and others 2010).
1097	However, documentation and descriptions of the technology principle, benefits, and food
1098	applications are scarce. Other concepts have also emerged, such as the technology applied
1099	in the emitter Verifrais™ (SARL Codimer, Paris, France). The active ingredients in the
1100	Verifrais $\degree$ system are sodium bicarbonate and ascorbic acid (Rooney 1995; Kerry 2014).
1101	There are also examples of (commercial) combined $O_2$ scavengers and $CO_2$ emitters on the
1102	market, such as Ageless $^{ extsf{@}}$ G (Mitsubishi Gas Chemical Co., Japan) and FreshPax $^{ extsf{@}}$ M
1103	(Multisorb Technologies Inc, USA). These systems are based on either ferrous carbonate or a
1104	mixture of ascorbic acid and sodium bicarbonate (Coma 2008).
1105	

One of today's most well documented CO<sub>2</sub>-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<sub>2</sub>-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<sub>2</sub>-emitter (non-commercial) on, for example, the microbial and sensory shelf life of cod 1111 fillets in MA-packages (70% CO<sub>2</sub>, 30% N<sub>2</sub>) and vacuum was investigated. The CO<sub>2</sub> level in the 1112 MA-packages (g/p ratio not given) with emitter decreased to approximately 40% after a few 1113 days of storage and thereafter increased and fluctuated around 50% CO<sub>2</sub> for the remaining 1114 storage time. For the vacuum-packages with emitter, the CO<sub>2</sub> level increased to 50% after 1115 one day of storage and reached 80% at day 15 (very limited headspace volume available). For the control (MAP and liquid absorber) the CO<sub>2</sub> level rapidly dropped to 20% after one 1116 1117 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 1118 1119 to 7 days for the control (MA-packaged with liquid absorber), supported by measured 1120 trimethylamine (TMA) levels and microbial analyses (total viable counts (TVC) and H<sub>2</sub>S-1121 producing bacteria).

1122

1123 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 1124 CO<sub>2</sub> and O<sub>2</sub> level and different g/p ratio. Packaging in modified atmosphere (60% CO<sub>2</sub>, 40% 1125 1126  $O_2$ ) with  $CO_2$  emitter at a low g/p ratio (1.3/1.0) resulted in extension of shelf life (14 – 21 1127 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 1128 1129 shelf-life obtained with the emitter was, however, comparable to that of cod packaged in 1130 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 1131 1132 storage time were different species of *Carnobacterium* and some *Photobacterium*. In the 1133 MA-packages with a g/p ratio of 1.3/1.0 including an emitter, the level of headspace CO<sub>2</sub>

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 <sub>2</sub> (g/p 1.6/1) was kept stable at about $35 - 37\%$ once the CO <sub>2</sub>
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 <sub>2</sub> 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	

The impact of the combined CO<sub>2</sub> emitter and liquid absorber (laboratory-type, custommade) on the quality and shelf life of fresh salmon has been thoroughly documented
(Hansen and others 2009a, b, c). Hansen and others (2009a) demonstrated the effectiveness
of a laboratory-type emitter in reducing the g/p ratio for MA-packages (60% CO<sub>2</sub>, 40% N<sub>2</sub>) of
salmon fillets without compromising the shelf life (based on microbial, sensory, and textural

1157 analysis). For the MA-packages (g/p 3/1) without emitter, the CO<sub>2</sub> level dropped to 40% 4 days after packaging and then stabilized. For the MA-packages (g/p 1/1) with emitter, the 1158 CO<sub>2</sub> level displayed an initial drop to about 45% (day 1), but subsequently the level increased 1159 1160 and reached 65 – 70% during the storage time. The measured TVC levels for the salmon 1161 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 1162 without emitter) reached a level of  $\log 5 - 6$  cfu/g after 15 days of storage, while for the 1163 1164 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 1165 1166 of fresh fish products with a significant reduction in package sizes and hence amount of 1167 packaging material, since a comparable shelf life can be obtained at significantly reduced g/p 1168 ratio.

1169

1170 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 1171 1172 fresh reindeer meat. The study documented prolonged sensory shelf life (odor evaluation) 1173 for meat packaged in modified atmosphere (60% CO<sub>2</sub>, 40% N<sub>2</sub>) with and without CO<sub>2</sub> emitter 1174 of 21 days, compared to 17 days for vacuum-packaged meat. For reindeer meat packaged in 1175 MAP with CO<sub>2</sub> emitter (storage temperature 15 °C), lower TVCs were measured after 13 and 1176 17 days ( $\log 3 - 4 \operatorname{cfu/g}$ ) 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 1177 same level of TVC (log 6 cfu/g) at the end of the storage time (day 21). The  $CO_2$  level in the 1178 1179 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<sub>2</sub>
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.

1185

In a similar study by Holck and others (2014) where the emitter capacity was more carefully 1186 1187 tuned towards the food product, the emitter compensated well for CO<sub>2</sub> absorbed by chicken fillets in MAP (100%  $CO_2$ ) and the drip loss was drastically reduced; from a weight loss of 1188 1189 7.5% for fillets packaged without emitter, to 2.5% for fillets packaged with an emitter in MA-1190 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<sub>2</sub> level 1191 1192 close to 100% throughout the storage time, while for the MA-packages without emitter the 1193  $CO_2$  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<sub>2</sub> with emitter and in 100% CO<sub>2</sub> 1194 1195 with regular fluid absorber. For the chicken packaged in 100% CO<sub>2</sub> with emitter, significant 1196 bacterial growth inhibition was detected; the bacteria required 7 additional days to reach a level of 10<sup>7</sup> cfu/cm<sup>2</sup> compared to packaging with commonly applied gas composition of 60% 1197 1198  $CO_2$  and 40%  $N_2$ . This study reports that packaging in 100%  $CO_2$  without emitter is not 1199 possible due to an unacceptably high drip loss.

1200

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

1203 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<sub>2</sub> generated in the vacuum-1204 1205 packages including an  $O_2$  scavenger/CO<sub>2</sub> emitter was reported to be 45% CO<sub>2</sub> after about 17 days of storage. The same CO<sub>2</sub> level was measured in the control vacuum-packages without 1206 emitter, speculated by the authors to be a result of  $CO_2$  emission by anaerobic 1207 microorganisms. The CO<sub>2</sub> level is unexpectedly low for vacuum-packages with O<sub>2</sub> 1208 1209 scavenger/ $CO_2$  emitter, considering the limited available volume and that the  $O_2$  level is 1210 stated to be zero. No information is provided on the total gas composition and the authors 1211 do not describe how the headspace gas composition in vacuum-packages was measured. 1212 Furthermore, it is not stated if the emitter capacity was product-adjusted and the concept of 1213 the technology is not clarified. In a different study, by Chen and Brody (2013), packaging with a CO<sub>2</sub>-releasing packaging structure (CSP Technologies, technology unknown) was 1214 1215 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 1216 meat product (cooked ham) stored at different temperatures and monitored over a 4-week 1217 1218 period. The percentage levels of  $CO_2$  gas in the vacuum-packages were measured as 56%, 1219 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 1220 1221 these bacteria from growing on the food product, rather than inhibiting the bacteria already 1222 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 1223 1224 are not included in Table 6 as the underlying technologies (active substances) of the emitters 1225 have not been published.

1226

Comparisons of different CO<sub>2</sub> emitter concepts are challenging. Differences in pre-handling 1227 of the food product, storage conditions and temperature, type of packaging material, g/p 1228 ratio, package size, and gas composition are all variables that will have an impact on the 1229 effect that the  $CO_2$  emitter contributes to the shelf life of the food product. Another 1230 important factor concerning the evaluation of CO<sub>2</sub> emitter that is rarely documented is the 1231 1232 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 1233 1234 amount and rate of  $CO_2$  release. Examples of materials applied in different layers of  $CO_2$ 1235 emitters/liquid absorbers are cellulosic fiber, or other fiber-based materials, SAP (super-1236 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 1237 1238 in the core of the substrate. These aspects should be taken into consideration when comparing the performance of different emitter systems. 1239 1240 1241 The results of earlier scientific studies, as summarized in the previous paragraphs, have

shown that an optimal effect of CO<sub>2</sub> 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<sub>2</sub> 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 1249 calculate the required amounts of sodium bicarbonate and citric acid, based on weight and 1250 surface area of the salmon fillets, g/p ratio, and tray capacity. Additional research is required 1251 on this topic focused on product-specific concepts, making CO<sub>2</sub> emitter technologies more 1252 flexible and suitable for a broader range of food products. 1253 With regard to commercialization, there are different emitters already on the market today. 1254 Emitters based on sodium bicarbonate and citric acid include CO<sub>2</sub> Freshpads (CO<sub>2</sub> 1255 Technologies, Urbandale, Iowa, USA)(Kerry 2014), SuperFresh (Vartdal Plastindustri AS, 1256 1257 Vartdal, Norway), and the Active CO<sub>2</sub> pad (CellComb AB, Säffle, Sweden). In addition, 1258 UltraZap® XtendaPak (Paper Pak Industries, La Verne, CA, USA), and the CO<sub>2</sub>Pad (McAirlaid's 1259 GmbH, Steinfurt, Germany) are based on other CO<sub>2</sub>-releasing concepts. 1260 1261 **Antimicrobial Packaging Systems** Antimicrobial food packaging presents a system designed to inhibit the growth of spoilage 1262 and pathogenic microorganisms. In this review, the most studied antimicrobial food 1263 1264 packaging systems have been classified according to their active substance/material: 1265 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, 1266 1267 antimicrobial nanoparticles are reviewed separately as the nano-size itself either increases or enables the antimicrobial activity (Table 11). 1268 1269

1270 Essential Oils (EOs)

1271 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 1272 1273 individual as well as for the environment (Alves-Silva and others 2013). Essential oils (EOs) 1274 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 1275 (Ruiz-Navajas and others 2013) and, as a result, EOs have been extensively studied as 1276 1277 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 1278 application to food. Some studies, however, have demonstrated the effectiveness of EO-1279 1280 enriched packages containing food and these are presented in Table 7.

1281

Cinnamon essential oil (CEO) is among the most studied EOs in active materials. Gherardi 1282 and others (2016) showed that a multilayer material containing about 18 and 10% of 1283 cinnamaldehyde as the major compound of the selected EO showed high activity against E. 1284 coli O157:H7 and S. cerevisiae, as the material reduced both microorganisms by 3 log 1285 1286 CFU/mL. Compared to the results obtained in culture media, E. coli showed higher sensitivity 1287 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 1288 1289 developed by incorporating a cinnamon essential oil/ $\beta$ -cyclodextrin inclusion complex into 1290 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 1291 1292 nano-film decayed on the eighth day compared to the third day for the control packed with 1293 fresh-keeping film. In this study, the initial bacterial load of the pork was 10<sup>3</sup>–10<sup>4</sup> CFU/g on

the first day and the unpacked control pork reached an excessive number of colonies after 4
days (above 1.10<sup>7</sup> CFU/g). Another option to control the release of major compounds of CEO
is to reversibly anchor cinnamaldehyde to a polymer, such as chitosan films, via iminocovalent bonding (Higueras and others 2015). The antimicrobial properties of chitosan-Schiff
base films in milk inoculated with *Listeria monocytogenes* led to a growth inhibition for 12
days under refrigeration conditions.

1300

1301 Carvacrol is another EO regularly used as a bio-based bioactive compound. However, the synergistic antimicrobial effect of different EOs on food has been less frequently studied. 1302 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 antimicrobial effect was observed with Botrytis cinerea, when the films were applied 1305 1306 through indirect contact with strawberries. The half maximal inhibitory concentration (IC50) of the EOs in the film was reduced from 40.4 mg/g (carvacrol only) to 13.2 mg/g (both EOs 1307 50:50). Knowing that the major volatile compounds of oregano EO is carvacrol, Rodriguez-1308 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 effective in inhibiting microbial growth. 1311

1312

To enhance safety and shelf life of cooked cured ham, Ruiz-Navajas and others (2015) studied 2 Spanish endemic species of thyme, *Thymus piperella* and *Thymus moroderi*. They reported that *T. piperella* had a higher effect than *T. moroderi*, probably due to the higher 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 1317 1318 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 1319 1320 mesophilic bacteria compared to the uncoated samples of T. piperella- and T. moroderibased films, respectively. In another study with thyme EOs, Quesada and others (2016) 1321 designed an active packaging system for the shelf-life extension of sliced ready-to-eat 1322 cooked pork during refrigerated storage. Interestingly, the package included an inner surface 1323 1324 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 1325 reported that yeast populations were affected by the presence of thyme EO and the yeast 1326 1327 counts decreased as a function of the EO dose in the film, especially during the first 21 days 1328 of storage.

1329

Arfat and others (2015) investigated microbiological and sensory changes of sea bass slices 1330 wrapped with fish protein/fish gelatin composite films incorporated with basil leaf essential 1331 1332 oil (BEO) during storage at 4 °C for 12 days. Films were incorporated with 100% BEO (w/w, 1333 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 1334 1335 vanillin have also been proposed as bioactive EOs. With the former, Llana-Ruiz-Cabello and 1336 others (2015) showed an efficiency against molds in lettuce during 7 days of storage (6.5% Proallium<sup>®</sup>). With the latter, Lee and others (2016) demonstrated that crab sticks packed 1337 with starfish gelatin films containing 0.05% vanillin exhibited antimicrobial activity against L. 1338 1339 monocytogenes previously inoculated on the food product.

1341	This brief overview illustrates that essential oil-based packaging has the potential to enhance
1342	food preservation. However, such packaging have not yet been extensively commercialized.
1343	Various factors need to be considered, such as the impact of EOs on (1) the organoleptic
1344	profile of the target food, (2) the physio-chemical properties of the materials, and (3) the
1345	effectiveness of this packaging system when manufactured under real conditions. In order to
1346	limit the constraint of their strong odor and taste, EO-based materials can be selectively
1347	used with compatible foods in terms of flavor. Another option could be the development of
1348	tasteless, colorless, and odorless EO derivatives (sensory inertness), such as some curcumin
1349	derivatives (Coma and others 2011; Etxabide and others 2016).
1350	
1351	According to the recent scientific publication by Dornic and others (2016), although EOs
1352	contain compounds naturally produced in the natural environment by higher plants, their
1353	consumption may nevertheless present a risk to health, given their composition. Indeed,
1354	their consumption may cause adverse effects when used inappropriately. The recent study
1355	of Rivaroli and others (2016) showed that higher doses of 3.5 g/animal/day could have a pro-
1356	oxidant effect in feedlot livestock. As mentioned by Eghbaliferiz and Iranshahi (2016),
1357	natural antioxidants can act as pro-oxidants, which produce free radicals and cause DNA
1358	damage and mutagenesis. Consequently, further research is needed to understand the
1359	potential toxicity of EOs incorporated into packaging materials.

#### 1360 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).

1364 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 1365 the glycosidic bonds of the Gram-positive bacterial peptidoglycans. Lysozyme incorporated into 1366 1367 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<sup>2</sup>, extending the shelf life of smoked salmon (Min and 1368 others 2005). Barbiroli and others (2012) reported incorporation of lysozyme and lactoferrin 1369 1370 into paper containing carboxymethyl cellulose, which allowed non-covalent binding of the 1371 positively charged proteins to the paper matrix. Tests on thin meat slices laid on paper sheets 1372 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 1373 with respect to the control. Lysozyme is accepted by the U.S. Food and Drug Administration 1374 (FDA 2001) as an antimicrobial agent in casings for frankfurters, and in Europe the use of 1375 lysozyme (E1105) falls under Directive 95/2/EC on food additives (European Union 1995). 1376

1377

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 1382 1383 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 1384 1385 example, packaging films coated with a cellulose derivatives-based solution containing 10,000 1386 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 1387 (Franklin and others 2004). Similarly, it was established that nisin-coated LDPE films were 1388 effective in inhibiting the bacterial flora in milk stored at 4 °C for 7 days, and the most 1389 1390 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 1391 1392 Directive 95/2/EC on food additives (European Union 1995).

1393

1394 The incorporation of nisin with other antimicrobial agents into PE and PE/polyethylene oxide films or polyamide coatings effectively inhibited Brochothrix thermosphacta, coliform bacteria 1395 growth and extended the shelf life of beef (Cutter and others 2001; Kim and others 2002) and 1396 fresh oysters (Kim and others 2002). According to the research of Khan and others (2016), the 1397 immobilization of nisin and EDTA on the surface of the cellulose nanocrystal/chitosan-films, by 1398 using genipin as a cross-linking agent, restricted the growth of psychrotrophs, mesophiles and 1399 1400 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. 1401 1402 monocytogenes in meat samples by 4.4 and 5.7 log CFU/g, respectively, after 35 days of 1403 storage. Furthermore, through the formation of nisin, citric acid, EDTA, and polyethylene glycol

sorbitan monooleate-coatings on polymeric films of different hydrophobicity (polyvinylchloride, 1404 1405 nylon or linear LDPE), the shelf life of refrigerated broiler drumsticks was extended by 0.6 to 2.2 1406 days (Natrajan and Sheldon 2000). In other studies, nisin, in combination with enterocins, 1407 sakacin, and potassium lactate, was incorporated into interleaves and tested on cooked ham 1408 and bacterial growth of L. monocytogenes (Jofré and others 2007) and Salmonella spp. (Jofré 1409 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), 1410 1411 and pediocin (Santiago-Silva and others 2009) have been incorporated into biopolymer-based 1412 films or used as coatings on various substrates, and they reduced bacterial (L. monocytogenes, Lactobacillus plantarum, Listeria innocua, Salmonella spp.) or fungal (Penicillium roqueforti) 1413 1414 growth on cooked ham, Wieners (2.5 log reduction), Gorgonzola cheese, and sliced ham (0.5-2 1415 log reduction), respectively.

1416

Further research effort is still needed to evaluate the release of enzymes and bacteriocins from 1417 various films and coatings into packaging, as well as diffusion to the surface of the food. 1418 1419 Moreover, the impact of the packaging on sensory properties of food should be thoroughly 1420 assessed. To date, only nisin and natamycin have been approved for use as food additives in 1421 various countries including the USA and the EU. Therefore, legislative issues regarding the use 1422 of bacteriocins as food preservatives remain the main limitation in their commercial exploitation. Nevertheless, the use of enzymes and bacteriocins in combination with other 1423 1424 preservation techniques can produce synergistic effects in food packaging while maintaining 1425 the safety and quality of minimally processed and fresh food products.

1426

### 1427 Antimicrobial Polymers

1428 Some polymers like chitosan or  $\epsilon$ -polylysine are inherently antimicrobial and are used in films 1429 and coatings (Table 9).  $\varepsilon$ -Polylysine is a natural antimicrobial polypeptide that is effective 1430 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 1431 (2010) developed  $\varepsilon$ -polylysine-containing whey protein films that significantly reduced the 1432 1433 specific growth rate of total flora and completely inhibited lactic acid bacteria growth in fresh-1434 cut beef portions as well as prolonged shelf life. 1435 1436 Chitosan, along with its derivative products (such as chitooligosaccharides), presents 1437 antimicrobial and antifungal activity against a wide range of target microorganisms, and it has 1438 also been proven to be beneficial to food packaging. Chitosan has been incorporated as an 1439 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) 1440 or used as a coating on plastic films (Joerger and others 2009). When chitosan-incorporated 1441

1442 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).

1454

1455 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 1456 efficacy of chitosan-coated plastic films incorporating 5 additional GRAS antimicrobials: nisin, 1457 1458 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. 1459 1460 monocytogenes, while the film containing sodium lactate was the most effective antimicrobial 1461 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. 1462 monocytogenes on cold-smoked salmon samples for at least 6 weeks (Ye and others 2008b). 1463 However, the authors indicated that sensory studies are needed before this technology is 1464 further developed (Ye and others 2008a). 1465

1466

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% 1470 1471 chitosan/5% LAE/2% sodium lactate/0.3% sorbic acid and 5% chitosan/5% LAE. Both 1472 combinations reduced S. typhimurium to an undetectable level at 0, 24, and 48 h, and 1473 significantly reduced L. innocua (even 6 logs after 48 h). Antimicrobial tests on surface-1474 contaminated turkey slices led to the reduction of *L. innocua* growth by 3 log CFU/cm<sup>2</sup> for both films. The films also reduced the growth of L. monocytogenes on the surface of ready-to-eat 1475 meat by 2.5-3 log CFU/cm<sup>2</sup> during storage of 3 or 5 weeks at 10 °C. For S. typhimurium the 1476 1477 reduction was  $1.5 \log CFU/cm^2$ .

1478

The effect of chitosan used in combination with nisin, potassium sorbate, or silver-substituted 1479 1480 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 1481 1482 aerobic mesophilic bacteria counts of samples packed in bags containing 2% of chitosan, nisin, 1483 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 1484 bags had lower TBARS values than those of samples in control bags. The exploitation of GRAS 1485 antimicrobials nisin and potassium sorbate in food packaging is straightforward, whereas the 1486 1487 use of silver zeolite as a surface biocide is debatable. Although it is approved by the US FDA as a 1488 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 1489 1490 were developed by incorporating lauric arginate ester (LAE) and their antimicrobial activity 1491 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, 1497 2011) for agricultural and medicinal purposes, but it is not yet specifically approved as an 1498 1499 antimicrobial food additive. Meanwhile, the other antimicrobial polymer mentioned above, 1500 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 1501 1502 a variety of other advantages, such as biodegradability, edibility, nontoxicity, biocompatibility, 1503 an aesthetic appearance, and good barrier properties. However, further studies are needed to 1504 fully evaluate industrial feasibility and the commercial viability of implementation of the 1505 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 1506 commercial food products held under real storage conditions. 1507 1508

# 1509 Organic Acids, their Derivatives and other Organic Compounds

1510 Some organic compounds such as selected organic acids and their derivatives, exhibit

antimicrobial activity (Table 10) and can be incorporated into packaging films.

1512

1513 Citric Acid. Júnior and others (2015) investigated the antimicrobial activity of citric acid on 1514 packaged minced beef. 30% of a mixture of citric acid/cornstarch/glycerol (ratio: 1.5:68.5:30) 1515 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 1516 1517 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 1518 identified the potential of active films containing citric acid to extend the shelf life of minced 1519 1520 beef. However, further research needs to be conducted to improve the limited antimicrobial effect demonstrated in this study. 1521

1522

1523 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 1524 1525 whiffiagonis) from microbial growth. The authors reported a positive antimicrobial effect 1526 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 1527 results obtained do not demonstrate a significant antimicrobial effect at the end of the shelf life 1528 (11 days), improved sensory properties (external odor, gill appearance, and odor) were 1529 reported for megrim packed with active films, while control samples were considered 1530 1531 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, 1532 inoculated with *L. monocytogenes* (10<sup>3</sup> and 10<sup>5</sup> CFU/g each), were wrapped in PVDC films 1533 1534 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<sup>5</sup> 1535 1536 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 1537 1538 inoculated L. monocytogenes nor that of mesophilic aerobic bacteria after 35 days of storage at 1539 4 °C. Beef samples inoculated with 10<sup>3</sup> 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 1540 control. Moreover, mesophilic aerobic bacteria and LAB counts in the beef packages with the 1541 1542 active films were found to be around 4 and 6 log lower than in control samples with initial L. monocytogenes inoculums of 10<sup>5</sup> and 10<sup>3</sup> CFU/g, respectively. This research, however, has 1543 some drawbacks, especially because the use of sorbic acid as an additive in meat products is 1544 1545 restricted according to Commission Regulation (EU) No 1129/2011 (European Commission 1546 2011). Sorbic acid can only be used for selected applications for meat products, such as aspic, 1547 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 1548 sample preparation, a solution-casting, laboratory-scale method, cannot be applied on an 1549 industrial scale. This trial should be repeated using melt (extrusion), however, the high 1550 temperature may influence the sorbic acid activity. 1551

1552

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. 1557 1558 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 1559 and 1.5% of each, applied in combination) in brine to protect smoked rainbow trout 1560 1561 (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 1562 bacteria counts were shown to be about 3, 2.1 and 1.7 log CFU/g lower for trout kept in 1563 1564 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 1565 and molds. Additionally, after 5 weeks of storage an identification of the bacteria species in the 1566 1567 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 1568 1569 bacteria in smoked fish, was the dominating species. This indicates that potassium sorbate was 1570 effective against this pathogen as it was not present in the corresponding samples.

1571

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 1579 1580 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 1581 demonstrated toxicological and microbiological stability (migration of sulfites below 10 mg/kg 1582 1583 SO<sub>2</sub>, according to Brazilian regulation for plastic materials in contact with food; and microbial counts below 10<sup>6</sup> CFU/g, considered as the quality threshold by the authors) throughout the 20-1584 day storage period. The active film provided the conditions suitable for apple consumption up 1585 1586 to 12 days of storage at 8 and 12 °C complying with the microbiological contamination limit of 6 1587 logs CFU/g.

1588

1589 Oxidized regenerated cellulose. Sezer and others (2016) incorporated oxidized regenerated cellulose micro-particles (4% w/w) in poly(E-caprolactone (PCL) films and evaluated their 1590 1591 antibacterial activity on packed sliced salami inoculated with *L. monocytogenes* (10<sup>4</sup> CFU/g). After 14 days of storage at 4 °C in contact with the active PCL films, 50% of total colony-forming 1592 1593 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 1594 regenerated cellulose micro-particles reduced the oxygen and water permeability by 93 and 1595 70%, respectively. 1596

1597

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
1601 combination with MAP (49%  $CO_2/0.5\% O_2/50.5\% N_2$ ), to catfish fillets stored at different 1602 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 1603 1604 catfish fillets from 4 to 5 days (18 µg AITC/L), 11 (36 µg AITC /L) and 23 days (MAP combined 1605 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 1606 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 1607 1608 combination of both technologies was not as effective as at 8 °C, but still extended the shelf life 1609 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 1610 1611 analysis of the final product should be performed to assure the acceptability of the product. In 1612 the context of odor, Kim and others (2015) recommend the application of AITC in vapor phase 1613 and low concentration (0.02-2500 mg/mL) to avoid negative impact on food. 1614 Commercial packaging solutions containing AITC can be found in a variety of formats (sheets, 1615 labels and films) on the Japanese market under the trademark Wasaouro<sup>™</sup> (Mitsubishi-Kagaku 1616 1617 Foods Corporation, 2002). However, even though antimicrobial tests with AITC (occurring in 1618 mustard) were successful on several types of food products (Kim and others 2015) and it has 1619 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 1620

1621 for AITC in EU and USA). In 2010, the EFSA panel on food additives and nutrient sources added

1622 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 1623 1624 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 1625 1626 (EFSA ANS Panel, 2010). To give another example, for sorbic acid, and its derivatives, such as 1627 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 1628 organic compounds are regulatory requirements. Therefore, research efforts should be focused 1629 1630 on the development of tailor-made active packaging solutions that comply with the specific legislation for each food product. 1631 1632 1633 Nanoparticles Antimicrobial nanomaterials represent an increasingly important component of some active 1634 1635 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 1636 1637 of packaged food. High surface-to-volume ratio and enhanced surface reactivity of the nanosized antimicrobial agents cause inactivation of microorganisms more effectively than their 1638 micro or macro-scale counterparts (Radusin and others 2016). The preparation of food 1639 1640 packaging materials depends on the nature of the nanoparticle, its size, and its specific surface 1641 area. 1642 1643 Despite the large number of studies reported in the literature in this area, there are only a few

1644 studies incorporating real food systems. Commonly used or tested antimicrobial nanoparticles

1645 are metal ions (silver, copper, gold, platinum), metal oxide (titanium dioxide, zinc oxide, 1646 magnesium oxide), and organically modified nano-clays. From ancient times, silver (Ag) has 1647 been used as an antimicrobial agent. Its ability as an antimicrobial agent increases in nano-1648 dimension and, hence, there are now many studies with Ag nanoparticles incorporated in food 1649 packaging materials as antimicrobial agents (Panea and others 2014; Azlin-Hasim and others 1650 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 1651 1652 nanoparticles, can significantly prolong the shelf life of different foodstuffs. Li and others (2017) reported that rice stored in LDPE without Ag/TiO<sub>2</sub> showed a serious mildew condition after one 1653 month with increased total plate counts (TPC) from 4.84 to 7.15 log cfu/g, while the rice stored 1654 in a nanocomposite based on LDPE with Ag/TiO<sub>2</sub> had a low TPC of 5.48 log cfu/g. Mihaly 1655 Cozmuta and others (2015) reported that the microbiological safety of bread stored in Ag/TiO<sub>2</sub>-1656 1657 based packaging inhibited the proliferation of yeast/molds, B. cereus, and B. subtilis. The shelf 1658 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. 1659 1660 Azlin-Hasim and others (2016) prepared nanocomposite material based on PVC and silver 1661 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) 1662 1663 reported reduction in MO but with higher lipid oxidation.

1664

1665 Emamifar and others (2010) conducted a study on the antimicrobial activity of LDPE loaded
 1666 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.

1672

1673 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 1674 1675 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 1676 log CFU/g, whereas those for the control sample increased from 2.1 to 3.37 log CFU/g (Bodaghi 1677 1678 and others 2013). In addition, copper (Cu) was effective against *Pseudomonas* spp. (isolated 1679 from spoiled fiordilatte cheese) when incorporated in PLA and used for packaging of fiordilatte 1680 cheese. A delay in microbial proliferation was recorded when the active films were used (Conte 1681 and others 2013).

1682

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).

1692

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.

1700

1701 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 1702 1703 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 1704 desirable and undesirable. A suitable antimicrobial active agent should be selected with respect 1705 to all these characteristics. In particular, the antimicrobial spectrum and the efficiency of the 1706 1707 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 1708 non-porous surfaces (ISO 22196 2011), derived from Japanese Industrial Standard (JIS Z 2801 1709 2000), a decrease of the number of microorganisms in the magnitude of 2 log colony forming 1710

units (CFU)/cm<sup>2</sup> is required to demonstrate antimicrobial efficacy. In food systems, shelf-life 1711 1712 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 1713 1714 specific and depends on several factors, such as the type of microorganisms (spoilage or 1715 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 1716 the Commission Regulation (EC) No 2073/2005) (European Commission 2005). For some food 1717 1718 systems, such as several bakery products, no visual mold growth should be observed, whereas 1719 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 1720 1721 since the agent may be entrapped or deactivated by the food component, or the activity of the 1722 agent may be affected by a low or high pH.

1723

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.

1731

1732 Conclusion

1733 Extensive research on the development of new active packaging technologies has been 1734 conducted over recent years generating a wide variety of active packaging systems that may be 1735 applied to extend the shelf life of food products. This review highlights the huge potential of 1736 active packaging systems and concludes that challenges in the implementation of new 1737 technologies to real food applications are similar across all the active packaging technology 1738 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 1739 1740 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 1741 1742 the food, packaging size, and storage conditions. Scale up and industrialization of the active 1743 packaging technologies could be challenging and therefore should be taken into consideration 1744 at early development state for successful commercialization. The cost of the implementation of 1745 the technology has to correspond with the benefit gained by the particular food product, 1746 legislative and regulatory issues must be addressed, and broad consumer acceptance is 1747 required. A successful collaboration between research institutes and industry, including 1748 development, legislative and commercial functions, is required to overcome these challenges. 1749 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 1750 1751 technologies and, hence, assist in accelerating their commercial adoption.

1752

## 1753 Acknowledgements

- 1754 All the authors participated in the "COST" Action (European Cooperation in Science and
- 1755 Technology) FP1405 "Active and intelligent (fiber-based) packaging innovation and market
- introduction", and they gratefully acknowledge the support provided which enabled the
- 1757 collaboration between the authors. The authors would also like to thank Stella Cook-Gummery
- 1758 of the Zurich University of Applied Sciences for her proofreading.
- 1759

## 1760 Author Contributions

- 1761 The authors made the following contributions to the manuscript:
- 1762 Selcuk Yildirim: Corresponding author. Development of concept of manuscript and distribution
- 1763 of work, interim and final revision, Abstract, Introduction, Conclusion, Oxygen scavengers,
- 1764 Bettina Röcker: Coordination of authors' contributions. Abstract, Introduction, Conclusion,
- 1765 Oxygen scavengers, Moisture scavengers, References
- 1766 Marit Kvalvåg Pettersen: Carbon dioxide emitters
- 1767 Julie Nilsen-Nygaard: Carbon dioxide emitters, Moisture scavengers
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- 1770 bacteriocins, polymers)
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- 1773 derivatives and other organic compounds)
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- 1775 (organic acids, their derivatives and other organic compounds)

1776 Veronique Coma: Antimicrobial packaging systems (essential oils)

1779

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## 2591 **Tables**

## 2592 See separate file