

Active Packaging Applications for Food

Selçuk Yildirim^{a*}, Bettina Röcker^a, Marit Kvalvåg Pettersen^b, Julie Nilsen-Nygaard^b, Zehra Ayhan^c, Ramune Rutkaite^d, Tanja Radusin^e, Patrycja Suminska^f, Begonya Marcos^g, Veronique Coma^h

^a *Zurich University of Applied Sciences, Institute of Food and Beverage Innovation, Department of Life Sciences and Facility Management, 8820 Wädenswil, Switzerland.*

^b *Nofima - Norwegian Institute of Food, Fisheries and Aquaculture Research, 1430 Aas, Norway.*

^c *Sakarya University, Faculty of Engineering, Department of Food Engineering, Serdivan, Sakarya, Turkey.*

^d *Kaunas University of Technology, Faculty of Chemical Technology, Department of Polymer Chemistry and Technology, 50254 Kaunas, Lithuania.*

^e *University of Novi Sad, Institute of Food Technology, 21000 Novi Sad, Serbia.*

^f *West Pomeranian University of Technology, Faculty of Food Sciences and Fisheries, Center of Bioimmobilization and Innovative Packaging Materials, 71-270 Szczecin, Poland.*

^g *IRTA, Food Technology, Finca Camps i Armet s/n, 17121 Monells, Spain.*

^h *Bordeaux University, UMR CNRS 5629, LCPO, 33607 PESSAC cedex, France.*

**Corresponding author. Email: selcuk.yildirim@zhaw.ch, Tel.: 0041 58 934 56 31*

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39 **ABSTRACT**

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

60

61 **Keywords:** active packaging, oxygen scavenger, antimicrobial packaging, antioxidant

62 releaser, ethylene absorber.

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64 END PAGE 2

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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	CPP	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 ^α -dodecanoyl-L-arginate or lauric arginate ester
91	LDPE	low-density polyethylene
92	LLDPE	linear low-density polyethylene
93	MA	modified atmosphere
94	MAP	modified atmosphere packaging
95	MDA	malonaldehyde
96	MO	microorganism
97	MRE	meal-ready-to-eat
98	OPET	oriented polyester
99	OPP	oriented polypropylene
100	OS	oxygen scavenger
101	OTR	oxygen transmission rate
102	PBAT	poly(butylendipate 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 _x	silicon oxide
116	SP/IP	smart and intelligent packaging
117	TBA	thiobarbituric acid
118	TBARS	thiobarbituric acid-reactive substances
119	TCC	total coliform counts
120	TiO ₂	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		

127

128 **Introduction**

129 Packaging plays critical role in the food supply chain. The primary function of packaging is to
130 serve as a container for the food enabling efficient transport within the whole supply chain,
131 preventing any physical damage, and protecting against manipulation and theft. Packaging
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
134 packaging acts as a barrier to protect the food from environmental influences such as
135 oxygen, moisture, light, dust, pests, volatiles, and both chemical and microbiological
136 contamination (Coles and others 2003; Yildirim 2011; Arvanitoyannis and Oikonomou 2012;
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
140 which highly gas permeable or perforated packaging materials are used to allow gas
141 exchange through the packaging (Lee and Paik 1997; Hussein and others 2015). Such
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
144 content of food has received much attention. There is an increasing trend to natural high-
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
149 packaging (MAP) (Ohlsson and Bengtsson 2002; Rodriguez-Aguilera and Oliveira 2009;

150 Sandhya 2010; Cooksey 2014; Zhuang and others 2014), active packaging (AP) (Singh and
151 others 2011; Yildirim 2011; Arvanitoyannis and Oikonomou 2012; Pereira de Abreu and
152 others 2012; Dobrucka and Cierpiszewski 2014; Realini and Marcos 2014; Kuorwel and
153 others 2015; Brockgreitens and Abbas 2016), smart and intelligent packaging (SP/IP) (Kerry
154 and Butler 2008; Lee and Rahman 2014; Realini and Marcos 2014; Biji and others 2015;
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
157 2014, Bumbudsanpharoke and others 2015). The emphasis of this review is on active
158 packaging.

159

160 Active packaging is an innovative approach to maintain or prolong the shelf-life of food
161 products while ensuring their quality, safety, and integrity. As defined in the European
162 regulation (EC) No 450/2009, active packaging comprises packaging systems that interact
163 with the food in such a way as to *“deliberately incorporate components that would release or*
164 *absorb substances into or from the packaged food or the environment surrounding the food”*
165 (European Commission 2009). Active packaging systems can be divided into active
166 scavenging systems (absorbers) and active-releasing systems (emitters). Whereas the former
167 remove undesired compounds from the food or its environment, for example, moisture,
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.

172

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

182

183 A large variety of active packaging systems has been developed and, to date, numerous
184 reviews have emphasized the potential of active packaging technologies to supply safer,
185 “healthier”, and higher-quality foods to the consumer (Kuorwel and others 2015;
186 Brockgreitens and Abbas 2016). However, the number of reviews presenting the benefits of
187 active packaging technologies applied to specific food products is limited (Llorens and others
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
190 applications. The complex structure of the food may influence the activity of the packaging.
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
193 bind to them, thereby inhibiting the desired activity. It is therefore important to critically
194 review active packaging studies pertaining to specific foods thereby enabling food and

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

197

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

204

205 **Oxygen Scavengers**

206 The application of oxygen scavengers (OS) is one of the main active packaging technologies
207 that aims to remove any residual oxygen present in the food package (Solovyov 2010;
208 Arvanitoyannis and Oikonomou 2012; Realini and Marcos 2014) or improve barrier
209 properties by acting as an active barrier (Sängerlaub and others 2013a). Several food
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
212 processes. However, the residual oxygen-concentration in the package often remains
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
215 during the packaging process, oxygen permeation through the packaging material or poor
216 sealing (Pereira de Abreu and others 2012), or due to oxygen dissolved in the food itself

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

219

220 Oxygen in packaging negatively affects the quality and shelf-life of several foods as it leads to
221 oxidation of the product (Choe and Min 2006) or promotes the growth of aerobic
222 microorganisms (Lee 2010; Solovyov 2010) resulting in color modifications (Møller and
223 others 2000; Nannerup and others 2004; Larsen and others 2006; Gibis and Rieblinger 2011;
224 Hutter and others 2016), or sensory changes (Jacobsen 1999; Granda-Restrepo and others
225 2009a; Li and others 2013), or nutritional losses (Chung and others 2004; Lopez-Gomez and
226 Ros-Chumillas 2010; Van Bree and others 2012). A reduction in the residual oxygen level of a
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
229 are iron-based scavengers (Miltz and Perry 2005; Galotto and others 2009; Braga and others
230 2010; Gibis and Rieblinger 2011; Polyakov and Miltz 2016), of which the OS activity is
231 triggered by moisture so that the reduced iron is irreversibly oxidized to a stable ferric oxide
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
234 palladium (Yildirim and others 2015), that catalyzes the oxidation of hydrogen into water.

235 Other systems that scavenge oxygen chemically include photosensitive dyes (Maloba and
236 others 1996; Miller and others 2003; Zerdin and others 2003; Perkins and others 2007),
237 ascorbic acid (Matche and others 2011; Pereira de Abreu and others 2012), gallic acid
238 (Wanner 2010; Ahn and others 2016), or unsaturated fatty acids (Arvanitoyannis and
239 Oikonomou 2012; Pereira de Abreu and others 2012). Moreover, there are also biochemical

240 mechanisms that work through the use of enzymes (Andersson and others 2002; Fernández
241 and others 2008; Kothapalli and others 2008; Nestorson and others 2008; Gohil and Wysock
242 2014), or biological approaches using bacterial spores (Anthierens and others 2011), or
243 yeasts that are immobilized in a solid material (Edens and others 1992). Some commercially
244 available oxygen-scavenging solutions are SHELFPLUS® O2 (OS-masterbatch, Albis Plastic
245 GmbH, DE), AMOSORB™ ColorMatrix™ (different OS-solutions, PolyOne™, Europe Ltd., UK),
246 Cryovac® (OS-film, Sealed Air Corporation, USA), AGELESS OMAC® (OS-film, Mitsubishi Gas
247 Chemical Inc., USA), OxyRx® (OS-containers, Mullinix Packages Inc., USA), or Aegis® OXCE
248 (OS-masterbatch, Honeywell International Inc., USA).

249

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

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

268

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

276

277 Military rations constitute a range of products in which a long shelf-life is of particular
278 interest. These food products are critical because the military requires good stability for a
279 minimum of 3 years without refrigeration (Gomes and others 2009) which presents a
280 challenge, especially with components with high oil content that are susceptible to oxidative
281 deterioration. Within this context, Gomes and others (2009) investigated the influence of an
282 iron-based OS-containing laminate material for its ability to extend the shelf-life of a hot-
283 filled meal-ready-to-eat (MRE) cheese spread, a component of military operational rations.
284 The authors demonstrated that the proposed O₂-absorbing laminate reduced the initial
285 headspace oxygen concentration in MRE pouches from 20.4 to 6.82 vol.-% (67.44 vol.-%

286 decrease) within the first 24 h, was further reduced to below 1 vol.-% within 11 days of
287 storage, and it remained below this level throughout the whole storage period (1 year). The
288 oxygen concentration in the regular MRE pouches also decreased by 50% during the first
289 15 days and remained constant at a concentration of 5 vol.-%. This oxygen decrease,
290 however, was assigned to the oxidative degrading reactions of the food. After 1 year of
291 storage, the positive effect of the O₂-absorbing laminate was illustrated through MRE cheese
292 spread with significantly lower rancidity and higher sensory acceptance compared to MRE
293 stored in packages without OS. Furthermore, the vitamin C content of the samples in the O₂-
294 absorbing laminate could be better preserved resulting in an almost 1.5 times higher
295 vitamin C content compared to the control samples. Thus, the authors clearly demonstrated
296 that the O₂-absorbing laminate removed oxygen before it was otherwise available for
297 degrading reactions in the food product.

298

299 A novel iron-based oxygen scavenger using iron nanoparticles, blended with activated
300 carbon, sodium chloride, and calcium chloride, was produced and evaluated by Mu and
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
303 scavenger-containing microscale pure iron powder (about 20 µm; estimated 1.8 mL/d).
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
306 utilized to inhibit lipid oxidation in lipid-containing foods. Storage of roasted sunflower seeds
307 and walnuts over a period of 120 days showed that the iron nanoparticles were an effective
308 means of inhibiting lipid oxidation. While the peroxide values (PV) of the control samples

309 without OS increased (sunflower seeds: from 4.32 to 46.89 meq O₂/kg oil, walnuts: from
310 2.41 to 21.85 meq O₂/kg), the PV of the samples containing the nano-sized oxygen scavenger
311 were shown to be significantly lower (sunflower seeds: 19.82 meq O₂/kg, walnuts: 8.84
312 meq O₂/kg) after 120 days of storage. Similar results were obtained for secondary lipid
313 oxidation. Although significant differences in the *p*-anisidine values (AnV) between the
314 samples were only observed after 40 and 80 days of storage of sunflower seed and walnut
315 samples, respectively; the AnV of the samples with the scavenger were about half that
316 without OS after 120 days. Nevertheless, the use of nanosized iron powder may further
317 reduce consumer acceptance due to possible leakage and unintentional consumption.

318

319 In some cases, preservation of food quality can be affected even if high barrier and modified
320 atmosphere packaging are applied, for example, if the sealing layer of packages exhibits
321 defects, particularly when these defects have a critical size that is below the detection limit
322 of standard leak testers of 10 µm (Sasaki and Kamimura 1997; Sänglerlaub and others
323 2013a). In this context, Sänglerlaub and others (2013a) simulated food packages with pinhole
324 defect sizes of 10 µm. They performed long-term storage experiments (300 days) to
325 compare O₂ absorption with a snack food product, salami in a baked bread roll, in packages
326 with and without an iron-based multilayer OS film. Although reactions of the food with
327 oxygen could not be fully prevented, oxidation reactions were significantly reduced by the
328 application of the OS film. Salami samples packed with OS showed less difference in color
329 (ΔE of 5.4) compared to those packed without OS (ΔE of 6.9). Additionally, the use of OS film
330 led to less lipid oxidation in the product resulting in hexanal concentrations almost 4 times
331 lower than those in the control samples without OS. Hence, the applicability of an oxygen

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

334

335 **Ascorbic Acid.** Matche and others (2011) modified LLDPE films by blending them with iron
336 and ascorbic acid (Fe/AA) or zinc and ascorbic acid (Zn/AA). Thereby, the ascorbic acid (AA)
337 acted as a reducing agent and the transition metals were used to catalyze the oxidation
338 reaction (Graf 1994). The incorporation of these reactive chemicals led to active films with
339 an OS performance of 47.6 and 37.4 mL, respectively, in 750 hours. With the application of
340 the OS films in the form of sealed bags to bakery products, an overall shelf-life extension of
341 packaged buns and bread slices could be demonstrated. In particular, the study of Matche
342 and others (2011) showed that microbial growth was retarded from 2 to 5 days in samples
343 packaged with the OS film. Instrumental texture analysis and moisture analysis revealed that
344 both bun and bread samples without OS film were significantly firmer and dryer,
345 respectively, after 4 days. This was explained by the lower water vapor transmission rates
346 (WVTR) of both Fe/AA (17.2) as well as Zn/AA films (17.4) compared to the pure LLDPE film
347 ($20 \text{ g/m}^2 \text{ 100 gauge/day}$), which was used as a control. Moreover, both modified variations
348 showed lower oxygen transmission rates (OTR). Sensory testing additionally supported the
349 obtained measured data as the bread slices and buns packed in OS films were sensorially
350 acceptable (softness and taste) up to 5 and 6 days, respectively, whereas the control
351 samples were not acceptable anymore on the second day. Despite these positive results, the
352 study lacks information about the package size and volume as well as the evolution of the
353 headspace oxygen concentration of the packaged bakery products. This hinders the

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

356

357 **Photosensitive dyes.** Maloba and others (1996) used a photosensitive OS film to improve
358 oxidative stability of sunflower oil. The applied ethyl cellulose polymer film contained the
359 common organic dyes eosin and curcumin and the synthesized polyether polyfuryloxirane
360 (PFO). When exposed to light, this OS film uses energy transfer to convert triplet oxygen
361 ($^3\text{O}_2$) into highly reactive singlet oxygen ($^1\text{O}_2$), which is absorbed irreversibly by the PFO. The
362 method and mechanism behind such systems are explained by Rooney (1995). The
363 sunflower oil was stored in the presence of the OS film and oxidative stability was evaluated
364 by determination of peroxide values (PV) and gas-chromatographic (GC) measurement of
365 headspace hexanal. To evaluate the influence of illumination, an initial irradiation period of 2
366 days at 2000 lux was followed by continuous illumination at normal room light (500 lux), the
367 latter imitating the light level commonly encountered on retailer's shelves. Control samples
368 were additionally stored under light exclusion. It was shown that sunflower oil stored under
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
371 lower with about 20 meq/kg (OS film, illuminated) compared to about 65 meq/kg (no OS
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
374 headspace hexanal indicating a high correlation ($r > 0.95$) between the 2 methods for
375 measuring rancidity. Thus, the OS film showed the potential to be applied with a wide range
376 of foods that contain polyunsaturated oils and that are stored under illumination at the

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

381

382 A similar OS film has also been successfully implemented with orange juice to protect juice
383 from oxidative degradation. Zerdin and others (2003) performed a storage study (1 year)
384 with orange juice filled in vacuum-sealed OS pouches. The OS activity of these pouches was
385 triggered by UV illumination just prior to packaging. The authors demonstrated that at 25
386 and 4 °C, the initial dissolved oxygen concentration of 2.7 ppm in the orange juice samples
387 packed in OS pouches was reduced to below 0.04 ppm within 3 and 7 days, respectively. In
388 contrast, for the control pouches without OS, dissolved oxygen in the orange juice was
389 above 0.04 ppm up to at least 35 and 77 days, respectively, clearly reflecting the impact of
390 the competition for the oxygen between the OS film and the ascorbic acid as well as the
391 impact of temperature. This was confirmed by the correlating ascorbic acid retention which
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)
394 and 51.3% (4 °C) after one year of storage. Furthermore, it was shown that the loss in
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
397 storage period, indicating freshly pressed orange juice (Johnson and others 1995). Storage at
398 25 °C led to an increased browning, however, the browning index of samples in the OS
399 pouches was significantly lower with about 0.34 compared to that of the control which was

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

405

406 In dairy products such as probiotic yogurt, the application of oxygen scavengers can be of
407 particular interest since dissolved oxygen has a negative effect on the viability of probiotic
408 bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium* spp. which are essential for
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
411 substituted anthraquinone (Rooney 1999). Unlike the OS film of Maloba and others (1996),
412 this film did not require a constant source of light. It only required UV light exposure to
413 trigger the scavenging process. Miller and others (2003) tested different manufacturing
414 methods of probiotic yogurt as well as different packaging systems, and they evaluated their
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
417 with an OS film. The initial dissolved oxygen concentration of 16 ppm was decreased to
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
420 observed within the first day was highlighted by the authors to be of particular importance
421 regarding the probiotic bacteria which require low oxygen concentration for post-
422 fermentation, thereby leading to a product with increased health benefit. In practical

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

425

426 In ultra-high temperature (UHT) milk, oxygen scavengers have been shown to prevent the
427 development of stale flavor. Perkins and others (2007) packaged indirectly processed UHT
428 milk using packaging films containing a prototype ZerO₂TM OS film laminate. Thereby, the OS
429 film significantly reduced the initial dissolved oxygen content of ~7 mg/L to ~3.8 mg/L during
430 14 weeks of room temperature storage (26 °C), compared to the control with ~5 mg/L.

431 Significant reductions of 23-41% were also observed for stale flavor volatiles such as methyl
432 ketones and aldehydes. Regarding lipid oxidation, a gradual increase in total free fatty acid
433 levels was observed during the 14-week storage period of samples without OS (data of OS
434 not published). However, the levels remained far below threshold values, indicating low
435 lipolytic rancidity. As a consequence, the consumer panel failed to detect a significant
436 difference in odor between the samples with and without OS. The authors concluded that
437 sensory analysis would have better reflected the rancid off-flavor, however, a lack in
438 regulatory approval for the OS prototype used in their study precluded taste-testing.

439

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

446 model system simulating citrus juice. With a 16-week storage period at 5 and 35 °C, the
447 authors demonstrated that the use of OS bottles could significantly slow down the
448 degradation kinetics of ascorbic acid and the browning reactions. Compared to glass jars and
449 conventional PET bottles, the vitamin C loss in the OS bottles was half at 35 °C storage and
450 even 3-4 times lower at the usual refrigerated storage of 5 °C. The authors reasoned the
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
453 demonstrated that glass containers could be advantageously replaced with polymeric
454 bottles including an oxygen scavenger, particularly in the case of beverages containing
455 ascorbic acid. However, the application of a real fruit juice instead of a model system would
456 have been preferable. Using the above-mentioned OS polymer in the form of a cast-
457 extruded monolayer-PET film, Galdi and Incarnato (2011) demonstrated the prevention of
458 the browning of bananas. Fresh-cut banana slices were shown to have significantly less
459 (~50%) color difference (ΔE) after three days wrapped in the OS film compared with the
460 conventional PET film. Later optimizations of these OS PET films resulted in co-extruded
461 multilayer films where the internal active layer was protected from fast oxidation by 2
462 external layers of pure PET (PET/OS-PET/PET) in order to increase the reaction time (Di Maio
463 and others 2015).

464

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

469 hours, even at very low oxygen levels (0.5 until 0.1 vol.-%), depending on the
470 product/headspace ratio (Andersen and Rasmussen 1992; Møller and others 2000;
471 Nannerup and others 2004; Larsen and others 2006; Gibis and Rieblinger 2011; Böhner and
472 others 2014; Hutter and others 2016); and most OS systems require several days to remove
473 initial headspace oxygen (Matche and others 2011). Recently, a rapid OS system based on a
474 catalytic system with palladium (CSP) has been developed. Palladium was coated on
475 PET/SiO_x-films using magnetron sputtering technology (Lohwasser and Wanner 2005;
476 Yildirim and others 2010, 2015). This OS film was able to remove up to 2.5 vol.-% residual
477 oxygen in food packages if hydrogen was included in the modified atmosphere (MA) of the
478 packaging (Yildirim and others 2015). Due to its high efficiency, this film is particularly
479 suitable for food products which are very susceptible to oxygen and where the oxidation
480 reactions are very fast. Hutter and others (2016) showed that an implementation of this OS
481 film in packaging prevented the discoloration of cooked cured ham. The OS film removed the
482 2 vol.-% initial oxygen in the headspace (160 cm³) of MA-packed ham within 35 min after
483 packaging. In this way, the color of the ham could be preserved and discoloration prevented
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
486 hours of storage. The same OS film was applied to bakery products. For packaged breads,
487 mold growth is the key factor limiting shelf-life. In this context, Rüegg and others (2016)
488 applied the palladium-based OS film in MA packages of partially baked buns, toast bread
489 slices, and gluten-free bread slices. At normal and modified atmosphere without OS film,
490 visible mold growth was detected in all samples after 2 days with a simultaneous decrease in

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

493

494 Apart from its high efficiency, the applied CSP also has some limitations. First of all,
495 hydrogen is required, therefore, the application of the CSP is limited to products packed
496 under modified atmosphere. As hydrogen concentrations >5.7 vol.-% in nitrogen are
497 considered as flammable, a maximum amount of oxygen to be removed was suggested to be
498 2.5 vol.-% (Yildirim and others 2015). Another drawback is that the catalytic activity of the
499 palladium-based system may be inhibited or even inactivated by volatile sulfur compounds
500 present in the headspace of certain packaged food products (Röcker and others 2017). With
501 regard to the safety of palladium, the EFSA published a scientific opinion on the safety
502 assessment of the palladium metal and hydrogen gas for use in active food contact
503 materials. The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing
504 Aids (CEF) concluded that *“the active substances palladium and hydrogen do not raise a
505 safety concern for the consumer when used as an oxygen scavenger in packages for foods
506 and beverages at room temperatures or below. Palladium should not be in direct contact
507 with food and should be incorporated in a passive structure impermeable to liquids which
508 prevents the migration at detectable levels”* (EFSA CEF Panel 2014).

509

510 Implementation of OS technologies into food packaging operations is often challenging as OS
511 systems are sensitive to environmental conditions, such as temperature and pH (Galdi and
512 others 2008; Solovyov 2010; Damaj and others 2014), or require humidity (Solovyov 2010) or
513 UV-light (Rooney 1999; Miller and others 2003; Zerdin and others 2003) to trigger the

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

521

522 For the selection of a suitable OS system for a specific food application, factors influencing
523 the oxidation kinetics, such as storage temperature, humidity, pH of the food and possible
524 light exposure, should be considered. Information about initial headspace oxygen
525 concentration, headspace volume, barrier properties of the packaging, as well as the
526 minimum shelf-life, is also essential to determine the required OS capacity and OS rate.
527 Finally, the cost of the OS packaging has to correspond with the benefit provided to the
528 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

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

542

543 Moisture control strategies in packaging can be divided into categories, such as moisture
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
546 headspace), moisture prevention (by barrier packaging), and moisture elimination (by
547 applying a desiccant/absorber). Only the latter category can be considered active, whereas
548 moisture reduction and prevention are more passive strategies. Passive systems can include
549 those that lower the humidity without any active ingredients, such as micro-perforated films
550 (for example, Xtend® films, Israel) (Suppakul 2015), and other packaging materials that are
551 inherently hygroscopic, such as those that are 100% cellulose-based (as defined in (EC) No
552 450/2009 (European Commission 2009)). Humidity levels inside packages can also be
553 controlled through the appropriate selection of packaging materials with a high barrier
554 against water vapor.

555

556 Active moisture scavengers can be further distinguished into 2 main types: RH controllers
557 that scavenge humidity in the headspace, such as desiccants, and moisture removers that
558 absorb liquids (Brody and others 2001). The latter can be applied in the form of pads, sheets,
559 or blankets, and are typically placed underneath fresh products in different packaging
560 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
562 others 1999, Day 2008). Thereby, drip loss increases with storage time and by increasing the
563 exposed surface area, and longitudinal cutting of the muscle fibers (McMillin 2008). Such
564 pads are mostly composed of porous materials, polymers (PP or PE), foamed and perforated
565 PS sheets, or cellulose, combined with superabsorbent polymers/minerals/salts
566 (polyacrylate salts, carboxymethyl cellulose, starch copolymers, silica/silicates) (Ščetar and
567 others 2010). Moisture absorbing pads are not often considered to be active packaging.
568 According to the EU Guidance to the Commission Regulation (EC) No 450/2009, "*Materials*
569 *and articles functioning on the basis of the natural constituents only, such as pads composed*
570 *of 100% cellulose, do not fall under the definition of active materials because they are not*
571 *designed to deliberately incorporate components that would release or absorb substance.*"
572 However, moisture absorbing pads containing components that "*are intentionally designed*
573 *to absorb moisture from the food*" and can be considered as active packaging (European
574 Commission 2009). Absorbing pads can also be used in combination with antimicrobials (for
575 example, Dri-Fresh® Fresh-Hold™ ABM, Sirane, USA), pH control agents and/or carbon
576 dioxide generators/oxygen scavengers, to avoid certain shortcomings, such as odor
577 generation or leakage.

578

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

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

588

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

595

596 **Bentonite/sorbitol/calcium chloride.** One of the potential application of moisture
597 scavengers is packaging of mushrooms since the quality of the mushrooms is strongly
598 influence by the RH in the headspace. Humidity below 86% RH promotes moisture and
599 hence weight loss, mushroom senescence, and textural changes; on the contrary, 100% RH
600 promotes psychrophilic bacteria growth and also causes discoloration of mushrooms. The
601 optimal humidity in the headspace of packaging for mushrooms was found to be 96%
602 (Mahajan and others 2008). Mahajan and others (2008) investigated new packaging
603 concepts for fresh mushrooms (*Agaricus bisporus*) - a produce with a high moisture content
604 and short shelf-life (1-3 days at ambient temperature). In this study, combinations of fast-
605 absorbing (CaCl₂, KCl, and sorbitol, with moisture holding capacity 0.91 ±0.01 [g H₂O/g] in
606 120 h) and slow-absorbing moisture absorbers (bentonite/sorbitol, with moisture-holding

607 capacity 0.34 ± 0.02 [g H₂O/g] in 120 h), were tested (Table 3). The best combination of
608 absorbers was found to be bentonite/sorbitol/CaCl₂ in proportions of 0.55:0.25:0.2 g/g
609 desiccant, respectively. The study revealed that the moisture holding capacity of the
610 scavenger is dependent on the relative humidity; it increased from 0.51 to 0.94 g water per g
611 desiccant when the relative humidity was increased from 76% to 96%. A change in
612 temperature (from 4 to 16 °C) did not have a significant influence on the moisture-holding
613 capacity. A positive impact of desiccant usage was observed, namely a decrease in the
614 moisture condensation inside the packaging, and improved transparency of the packaging
615 film. Mushrooms (250 g) packed with 5 g of bentonite/sorbitol/CaCl₂ desiccants resulted in a
616 lower browning index (BI 14.8) compared to those packed without desiccants (BI 18) after 5
617 days of storage at 10°C. However, for packages with higher levels of desiccants (10 or 15 g),
618 browning indices were greater due to the browning and excessive moisture loss. The
619 appearance of mushrooms packed with 5 g desiccants was also better since higher amounts
620 resulted in excessive moisture loss. After 5 days, the mushrooms packed with 5 g of
621 desiccants were still sellable on the market. The results show that the capacity of the
622 moisture absorbers had to be precisely tuned and product-adjusted to achieve an optimal
623 effect, as too high absorption capacities could result in a decrease in quality. Similar
624 experiments were performed by Azevedo and others (2011). A mixture of desiccants
625 consisting of calcium oxide/calcium chloride/sorbitol in a ratio of 0.5:0.26:0.24, respectively,
626 resulted in a moisture-holding capacity of 0.81 g water per g of desiccant. However, no food
627 application was evaluated in this study.

628

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

643
644 **Sodium chloride and hygroscopic ionomer.** Langowski and others (2006) and Sangerlaub
645 and others (2013b) developed salt-embedded, humidity-regulating trays consisting of a 3-
646 layer structure: barrier layer, active layer with NaCl and sealing layer, to control the humidity
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
649 multilayer structure containing a foamed hygroscopic ionomer Entira™ AS SD100 as an active
650 layer, were used by Rux and others (2016) to pack tomatoes and strawberries (Table 3).
651 Thereby, the active layer contained 0 or 12 wt% NaCl. When just water was packed, the

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

671

672 **Tamarind seed galactoxyloglucan.** Polysaccharides, such as galactoxyloglucan from tamarind
673 seeds, can be used as moisture-absorbing aerogels. Such aerogel-based packaging systems,
674 in combination with enzyme-based (galactose oxidase) oxygen-scavenger systems, were

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

680

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

688

689 As described in this section, various moisture scavenging systems can be applied to preserve
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
692 most well-established and commercially well-recognized technologies incorporate the use of
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
695 in other forms, such as in the structure of packaging or as a coating, for commercial food
696 product applications (fresh fruits, vegetables, fish and meat) is still under development and
697 future research is expected to be focused in this area (Restuccia and others 2010).

698

699 **Ethylene Absorbers**

700 Ethylene (C₂H₄) is a growth-stimulating hormone (plant growth regulator) accelerating
701 ripening and senescence through increasing the respiration rate of fresh and minimally
702 processed climacteric produce and shortening the shelf-life during postharvest storage.
703 Ethylene also accelerates chlorophyll degradation rates, especially in leafy products, and
704 enhances excessive softening of fruits (Saltveit 1999; Ozdemir and Floros 2004). For these
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

709 **Potassium permanganate.** Ethylene scavenger systems involve either inclusion of a small
710 sachet containing an appropriate scavenger in the packaging or incorporation of an ethylene
711 absorber in the film structure. The sachet material should be highly permeable to ethylene,
712 allowing diffusion through it. The most commonly used active component of the sachet is
713 potassium permanganate (KMnO₄) in order to oxidize/inactivate ethylene (Floros and others
714 1997; Ayhan 2011; Llorens and others 2012) However, KMnO₄ is never used in direct food
715 contact due to its high toxicity (Martínez-Romero and others 2007).

716

717 **Minerals.** Another ethylene-scavenging system is based on the use of finely dispersed
718 minerals, such as zeolite, active carbon, or pumice. These minerals could be incorporated
719 into a plastic film structure commonly used in fresh produce packaging (De Kruijf and others
720 2002). Such minerals are intended to scavenge ethylene and also modify the gas

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

724

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

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

745

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

757

758 The incorporation of scavengers in packaging films may be a better option to solve sachet-
759 related problems. Ethylene scavengers could either be embedded into a solid, dispersed in
760 plastic, or incorporated into various layers of the packaging (Ozdemir and Floros 2004).

761 However, there has been only limited research into the application of ethylene absorbers in
762 the structure of packaging films. The main focus of the following section is the application of
763 ethylene scavengers incorporated into the actual packaging material, rather than in sachet
764 format, for fresh produce. Ethylene scavenger can prolong the shelf life of climacteric fruits,
765 such as apples, kiwifruit, apricot, bananas, mango, cucumber, tomato, and avocados, and

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

769

770 **Nano-particles.** Nano-TiO₂ is reported to oxidize ethylene into H₂O and CO₂ (Han and Nie
771 2004). Yang and others (2010) tested PE blended with nano powders of Ag, TiO₂, and kaolin
772 for preservation of fresh strawberries at 4 °C for 12 days. Results showed that active PE with
773 nano-powders maintained physicochemical and physiological quality and sensory attributes
774 of strawberry better than the control (PE). Active packaging decreased the rate of fruit decay
775 (to 16.7% for nano-packaging and 26.8% for normal packaging), maintained the content of
776 total soluble solids, preserved ascorbic acid, and reduced the malondialdehyde content (to
777 66.3 μmol/g for nano-packaging and 75.4 μmol/g for normal packaging), and enzyme activity
778 of polyphenol oxidase and pyrogallol peroxidase. However, the gas composition including
779 ethylene in the headspace was not monitored in this study and there is no indication of shelf
780 life.

781

782 Chinese bayberries were packaged by Wang and others (2010) with active PE including 30%
783 nano powder of Ag, TiO₂, and kaolin-clay or treated with hot air or the combination of hot air
784 treatment and nano-packaging, and stored at 1 °C and 80-90% RH for 8 days. Results showed
785 that the application of hot air (48 °C for 3 h) and/or active packaging reduced the incidence
786 of green mold decay (from 75.5% to 34.6% for active packaging and to 22.7% for hot air
787 treatment and to 14.8% for the combined treatment), fruit respiration, and ethylene
788 production, and maintained fruit firmness compared to the control (fruit directly packed in

789 PE with no heat treatment) for 8 days of storage. The respiration rate and ethylene
790 production of the combined treatment of hot air and active packaging were 49.6% and
791 25.9%, respectively, which were lower than the control. This study suggested that the
792 combined treatment was more effective in maintaining the quality of Chinese bayberries
793 than heat treatment or nano-packaging alone.

794

795 Li and others (2009) studied the effect of active packaging produced by blending nano
796 powders of nano-Ag, nano-TiO₂, and kaolin with polyethylene for the preservation of
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
800 evolution during 12 days of storage. An important index of rate of browning of the product
801 was reduced from 0.7 to a lower level of 0.6 on day 12. The ethylene production rate
802 increased initially and then declined for all treatments. The maximum ethylene content was
803 reported as 17.6 μL/kg h for the control on the third day and 9.2 μL/ kg h for nano-packaging
804 on the sixth day of storage. The active packaging is recommended for Chinese jujube to
805 improve quality, however, a specific shelf life was not indicated.

806

807 Hu and others (2011) studied the effect of PE blended with nano-Ag, nano-TiO₂, and
808 montmorillonite on the quality of ethylene-treated mature kiwifruit at 4 °C for 42 days.
809 Weight loss, softening, color variation, and Brix degrees (°Brix) of kiwifruit were significantly
810 reduced by 22.7%, 124.8%, 23.5% and 14.4%, respectively. Ethylene concentrations in the
811 headspace were 39.5 μL/L and 16.8 μL/L for the control and nano-packaging, respectively,

812 on storage day 42. For kiwifruit, 30 $\mu\text{L/L}$ was reported to cause unacceptable softening. The
813 researchers stated that nanocomposite packaging was effective in inhibiting ethylene
814 production (57.4% of lower headspace ethylene in active packaging), preventing
815 physiological changes, and delaying ripening, however, no specific shelf life was indicated. A
816 lower level of ethylene production was related to the synergistic effect of nanoparticles
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
820 enzyme activity, retarded ethylene production, maintained °Brix and titratable acidity
821 compared to uncoated PVC (control). Maximum ethylene content was reported as 40 $\mu\text{L/kg}$
822 day for nano-packaging on the ninth day and, 70 $\mu\text{L/kg}$ day for the control on the sixth day of
823 storage. The browning index was significantly reduced from 31.7 to 23.9 on day 12,
824 maintaining the initial appearance. The activity of polyphenol oxidase was 9.6 U/g min in
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
828 to have similar physical properties to TiO_2 which oxidizes ethylene into water and carbon
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-
833 dimensional structure with cation exchange, adsorption, and molecular sieving properties.
834 Therefore, zeolites have been used in many industrial and agricultural applications, including

835 as an ethylene-absorbing additive incorporated into packaging films. There are various
836 reports that incorporation of zeolites increases gas permeability of packaging films by means
837 of their crystalline porous-three-dimensional framework structure (Süer and others 1994;
838 Kittur and others 2005; Zhao and others 2011). Esturk and others (2014) successfully applied
839 low-density polyethylene (LDPE) bags with ethylene absorber (8% Tazetut® master batch, an
840 inorganic product containing 50% of various alumino-silicate minerals (zeolite)) to broccoli
841 florets under passive modified atmosphere and stored at 4 °C for 20 days. The authors
842 stated that spoilage occurred quickly in unpackaged broccoli (control) illustrated by
843 chlorophyll degradation, stem-hardening, and mass loss of 41.5% on day 20, which was less
844 than 1% for packaged applications. The product (control) was unacceptable for the sensory
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
847 0.33 ppm in active LDPE at the end of the storage. Thus, packaging with zeolite-based active
848 films extended the shelf life of broccoli up to 20 days, compared to a 5-day shelf-life for the
849 unpackaged product.

850

851 The quality of kiwifruit packaged with HDPE bags including a sachet with KMnO_4
852 impregnated zeolites at 4 °C for 31 days was reported by Küçük (2006). 0.2 mL KMnO_4 /g
853 zeolite was impregnated with zeolites of 1-3 mm in size. KMnO_4 impregnated zeolites were
854 added into the HDPE bags as 5 and 10% of the amount of kiwifruit. Fruits were firmer and
855 had a higher vitamin C content in zeolite-containing HDPE films compared to the control
856 (60.67 mg/100 mL of vitamin C for fruits packaged using HDPE with 5% zeolite and 47.37
857 mg/100 mL for the control on day 31). There was no significant difference reported for color

858 values L^* , a^* , and b^* (L^* indicating lightness, a^* chromacity on a green (-) to red (+) axis, and
859 b^* chromacity on a blue (-) to yellow (+) axis) between HDPE bags with and without zeolite.
860 The ethylene measurements for each treatment and the shelf life were not published in this
861 publication.
862
863 Boonruang and others (2012) tested 4 different packaging films for mango at 12 °C. The
864 tested materials were non-perforated, highly gas-permeable film, non-perforated highly gas-
865 permeable film with ethylene-absorbing property, micro-perforated highly gas-permeable
866 film and common low-density polyethylene film. The non-perforated film with ethylene
867 absorber extended the shelf life of mango to 40 days at 12 °C, compared to 35 days with the
868 non-perforated, highly gas-permeable film, 30 days with the microperforated film , and 5
869 days with the common low-density polyethylene. The film with ethylene absorber reduced
870 weight loss, maintained firmness, and there was no sign of decay during storage. Low
871 ethylene concentrations (<3.5 $\mu\text{L/L}$) were reported in mangoes with the different packaging
872 films. Ethylene production in the microperforated packages was higher than that in the non-
873 perforated and non-perforated with ethylene absorber packaging. The ethylene-absorbing
874 characteristics of the material delayed the ripening process of mangoes. A further study
875 shows that zeolite-added LDPE bags were applied to ripe kiwifruits successfully establishing
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)
878 during 20 days of cold storage at 4 °C. A minimum shelf life of 20 days was suggested for
879 kiwifruits using zeolite-incorporated LDPE bags. Ethylene measurement is not reported in
880 this study (Ayhan 2016).

881

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

893

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

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

907

908 Packaging materials integrated with ethylene-removers in the packaging structure are still
909 limited in commercial applications. The use of these materials for a broad spectrum of fresh
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
915 adequate oxygen and low carbon dioxide-modified atmosphere packaging combined with
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.
918 However, it should be noted that packaging parameters should be designed to be produce-
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**

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

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

931

932 Torres-Arreola and others (2007) reported a delay in lipid oxidation and protein
933 denaturation in fresh sierra fish fillets through the incorporation of BHT into LDPE packaging.
934 Compared to LDPE films, samples packed in BHT-LDPE films demonstrated lower lipid
935 oxidation, expressed as thiobarbituric acid-reactive substances (TBARS) values (4.20 ± 0.52
936 vs 11.95 ± 1.06 mg malonaldehyde (MDA)/kg), peroxide index values (7.20 ± 1.38 vs 15.15
937 ± 1.48 meq/kg), and free fatty acid contents (7.98 ± 0.43 vs 11.83 ± 1.26 of oleic acid). Fillets
938 packed in BHT-LDPE packaging films also inhibited less tissue damage and better retained
939 firmness than fillets packed in LDPE. However, the presence of synthetic antioxidants in food
940 is being questioned, therefore, the alternative approach that is being widely studied is the
941 use of natural antioxidants such as α -tocopherol. Poly(lactide-co-glycolide) films loaded with
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
944 have a higher volatility than α -tocopherol, therefore it was expected that they would be
945 more suitable for dry food applications. However, in this study, α -tocopherol offered the
946 same antioxidant protection for whole milk powder exposed to light and oxygen. In a further
947 study, sealable multilayer films (HDPE/EVOH/LDPE) manufactured with an inner LDPE layer
948 containing 4% of α -tocopherol delayed the lipid oxidation of whole milk powder at 30 and 40
949 °C (Granda-Restrepo and others 2009b). Similarly, sealable LDPE films containing 1.9 and 3%

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

962

963 Active antioxidant food packaging films produced by incorporating 4.6% of the natural
964 flavonoid quercetin into EVOH matrix showed enhanced lipid oxidative stability, as
965 demonstrated by a lower peroxide index (12 vs 27 meq/kg) and a reduction in TBARS values
966 by 25% during storage time (López-de-Dicastillo and others 2012b). Catechin, an active
967 component of green tea with properties similar to those of quercetin, was shown to be an
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
970 with active films containing 0.33 and 1.34% of catechin at 37 °C for 40 days resulted in a
971 strong reduction in hexanal content released into the headspace up to 25 days, after which
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
974 months. On exposing sunflower oil to the films, the peroxide values demonstrated that the
975 films actively protected the oil. Moreover, the films with quercetin (0.76 and 4.01%) were
976 more effective compared to those with catechin due to the higher solubility of quercetin in
977 this product, as well as its higher antioxidant capacity. The reported results using accelerated
978 shelf life testing (37 °C) to estimate the antioxidant potential of the films. However, the
979 antioxidant performance under real storage conditions needs to be evaluated before
980 commercial implementation. Another issue to be addressed would be the inclusion of EVOH
981 in a multilayer system in order to protect the system from water and to reduce costs, while
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
984 LDPE films demonstrated antioxidant activity when in contact with brined sardines and
985 ground beef (López-de-Dicastillo and others 2012b; Júnior and others 2015).

986

987 Antioxidant packaging systems containing volatile extracts, essential oils, or active
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
990 corn-zein-laminated linear LLDPE films were used for fresh ground beef packaging and
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
993 wt% of the phenolic antioxidant resveratrol, which is naturally produced by plants under
994 stress conditions, showed strong antioxidant activity, reduced lipid oxidation by 34.7%, and
995 extended the shelf life of fresh meat stored at 4 °C by a few days (Busolo and Lagaron 2015).

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, O₂:CO₂) 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

1019 stability of lamb and beef meat (Nerín and others 2006; Camo and others 2008, 2011).
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
1024 others (2011, 2016) developed LDPE films coated with rosemary extract that were able to
1025 protect meat patties from high-pressure-processing-induced lipid oxidation and
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 $11.83\pm 1.26\%$ 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
1033 has also been explored as a means of providing added value to these residues. Packaging of
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,
1039 beet root residue powder, and mango and acerola pulp incorporated into sealable
1040 biodegradable films had a protective effect on sunflower and palm oil oxidation (Souza and
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
1044 encapsulated anthocyanins presented lower values (4.7-28.7 meq/kg) (Stoll and others
1045 2016). Similarly, a lower peroxide index, which was significantly different from that of the
1046 control (oil with no packaging), was detected in palm oil packed in cassava starch films with
1047 high concentrations of mango and acerola pulp additives (Souza and others 2011). However,
1048 it was found that vitamin C in acerola pulp acted as a pro-oxidant agent, which suggests that
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
1053 the antioxidant packaging systems in real commercial food applications and do not consider
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.
1056 Research efforts should focus on the use of packaging materials obtained through scalable
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,
1059 industrial packaging techniques (such as MAP or vacuum vs wrapping), effect on sensory
1060 properties of food, and validation using real storage conditions.

1061

1062 **Carbon Dioxide Emitters**

1063 The antimicrobial effect of CO₂ is thoroughly documented in the literature (Kolbe 1882;
1064 Valley 1928; Haas and others 1989; Debs-Louka and others 1999). CO₂ is soluble in the

1065 aqueous and fatty phases of food products and the antimicrobial effect is highly dependent
1066 on the rate of solubility and amount of CO₂ dissolved in the food product. The solubility of
1067 carbon dioxide increases with decreasing temperature (Devlieghere and others 1998;
1068 Devlieghere and Debevere 2000) and also varies for different food products depending on
1069 the properties of the food such as surface area, pH and composition (water, fat, protein)
1070 (Chaix and others 2015). The antimicrobial effect has been found to be proportional to the
1071 partial pressure of the gas (Blickstad and others 1981). In terms of food packaging, this
1072 implies that the total amount of CO₂ present in the headspace of the package is crucial for
1073 the effect. The concept of a CO₂-releasing device to be implemented in modified
1074 atmosphere packages (MAP) to maintain high headspace levels of CO₂ during storage, and
1075 thereby, facilitate smaller package volumes (lower gas to product (g/p) volume ratio), and
1076 prolonged shelf life was introduced in the 1990s.

1077

1078 The implementation of CO₂ emitters in MA-packages may allow for increased filling degree,
1079 reduced package sizes, improved transport efficiency, and a net reduction in environmental
1080 impact. The release of carbon dioxide from a tuned emitter system may also prevent
1081 packaging deformation as it compensates for CO₂ absorption into the food product in the
1082 initial stages of storage. In this way it counteracts formation of negative pressure in MA-
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
1085 growth of spoilage bacteria and prolonged shelf life for fresh food products at sustained high
1086 CO₂ levels in the packages will have a knock-on effect in the form of a reduction in food
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 CO₂. Over the last decade, the
1092 field of CO₂ emitters has advanced significantly, reflected in increased research activity and
1093 sale of commercial CO₂ emitters. Table 6 lists the CO₂ 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 VerifraisTM (SARL Codimer, Paris, France). The active ingredients in the
1100 Verifrais[®] system are sodium bicarbonate and ascorbic acid (Rooney 1995; Kerry 2014).
1101 There are also examples of (commercial) combined O₂ scavengers and CO₂ emitters on the
1102 market, such as Ageless[®] G (Mitsubishi Gas Chemical Co., Japan) and FreshPax[®] 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

1106 One of today's most well documented CO₂-releasing systems is based on a combination of
1107 the active substances sodium bicarbonate and citric acid. Several scientific publications
1108 document the effect of this CO₂-releasing system. Amongst the first reports in the literature
1109 of such applications is a study from 1995 (Bjerkeng and others 1995). In this study the effect
1110 of CO₂-emitter (non-commercial) on, for example, the microbial and sensory shelf life of cod

1111 fillets in MA-packages (70% CO₂, 30% N₂) and vacuum was investigated. The CO₂ 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% CO₂ for the remaining
1114 storage time. For the vacuum-packages with emitter, the CO₂ level increased to 50% after
1115 one day of storage and reached 80% at day 15 (very limited headspace volume available).
1116 For the control (MAP and liquid absorber) the CO₂ level rapidly dropped to 20% after one
1117 day of storage. The cod fillets packaged in modified atmosphere and vacuum with emitter
1118 were found to have a sensory shelf life, based on ammonia-like odor, of 11 days, compared
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 H₂S-
1121 producing bacteria).

1122

1123 In a study by Hansen and others (2007), the effect of the same emitter system was
1124 investigated, also in the packaging of cod fillets, looking at the simultaneous effect of high
1125 CO₂ and O₂ level and different g/p ratio. Packaging in modified atmosphere (60% CO₂, 40%
1126 O₂) with CO₂ 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
1128 bacterial growth when compared to vacuum-packaging (7 – 14 days total shelf life). The
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
1131 with emitter and g/p 3.9/1.0 without emitter), the dominating bacteria at the end of the
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 CO₂

1134 increased to about 70 – 80% during the storage period, effectively compensating for the CO₂
1135 absorbed by the product, whilst for MA-packages with a g/p of 3.9/1.0 without emitter, the
1136 CO₂ 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 CO₂ 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% CO₂ and 40% N₂) and CO₂ emitter, based on sensory (odor and appearance assessment)
1141 and microbiological evaluation (TVC, H₂S-producing bacteria counts, lactic acid bacteria
1142 counts (LAB), and microbiota analysis). With a CO₂ emitter present in the MA-packages, the
1143 headspace level of CO₂ (g/p 1.6/1) was kept stable at about 35 – 37% once the CO₂
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 CO₂ 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 TVC of log 7.1 cfu/g, while the number for the
1148 cod in MA-packages and MAP with emitter was log 6.4 cfu/g and log 5.5 cfu/g, respectively.
1149 In the studies described above, the laboratory-type emitters were custom-made; the ratio
1150 between citric acid and sodium bicarbonate was adjusted to the pH of the food product.
1151
1152 The impact of the combined CO₂ emitter and liquid absorber (laboratory-type, custom-
1153 made) on the quality and shelf life of fresh salmon has been thoroughly documented
1154 (Hansen and others 2009a, b, c). Hansen and others (2009a) demonstrated the effectiveness
1155 of a laboratory-type emitter in reducing the g/p ratio for MA-packages (60% CO₂, 40% N₂) of
1156 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 CO₂ level dropped to 40% 4
1158 days after packaging and then stabilized. For the MA-packages (g/p 1/1) with emitter, the
1159 CO₂ level displayed an initial drop to about 45% (day 1), but subsequently the level increased
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
1162 the two packaging methods was the same. The TVC of the MA-packaged samples (with and
1163 without emitter) reached a level of log 5 – 6 cfu/g after 15 days of storage, while for the
1164 vacuum-packaged salmon, the same bacterial counts were measured 7 – 10 days into
1165 storage. The results illustrate that a CO₂ emitter can allow for a more sustainable packaging
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
1171 Pettersen and others (2014), the effect of different packaging methods was evaluated for
1172 fresh reindeer meat. The study documented prolonged sensory shelf life (odor evaluation)
1173 for meat packaged in modified atmosphere (60% CO₂, 40% N₂) with and without CO₂ emitter
1174 of 21 days, compared to 17 days for vacuum-packaged meat. For reindeer meat packaged in
1175 MAP with CO₂ emitter (storage temperature 15 °C), lower TVCs were measured after 13 and
1176 17 days (log 3 – 4 cfu/g) compared to MAP without emitter and vacuum-packaging at the
1177 same sampling times (log 4 – 5 cfu/g). Samples from the 3 packaging methods reached the
1178 same level of TVC (log 6 cfu/g) at the end of the storage time (day 21). The CO₂ level in the
1179 MA-packages with emitter displayed an initial decrease after 1 day of storage to 56%, but

1180 increased to 67% towards the end of the storage time. The packaging strategy with CO₂
1181 emitter also resulted in a significantly reduced drip loss for the reindeer meat; 1% for MAP
1182 with emitter compared to 3% for MAP without emitter. The article concluded that the
1183 capacity of the custom-made emitter was too low for the product, implying that the
1184 beneficial effect of an emitter could be expected to be more pronounced.

1185

1186 In a similar study by Holck and others (2014) where the emitter capacity was more carefully
1187 tuned towards the food product, the emitter compensated well for CO₂ absorbed by chicken
1188 fillets in MAP (100% CO₂) and the drip loss was drastically reduced; from a weight loss of
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
1191 packaging collapse and physical squeeze on the fillet. The emitter maintained the CO₂ level
1192 close to 100% throughout the storage time, while for the MA-packages without emitter the
1193 CO₂ level dropped slightly to 90 – 95% depending on the g/p ratio. The microbial shelf life
1194 was found to be the same for fillets packaged in 100% CO₂ with emitter and in 100% CO₂
1195 with regular fluid absorber. For the chicken packaged in 100% CO₂ with emitter, significant
1196 bacterial growth inhibition was detected; the bacteria required 7 additional days to reach a
1197 level of 10⁷ cfu/cm² compared to packaging with commonly applied gas composition of 60%
1198 CO₂ and 40% N₂. This study reports that packaging in 100% CO₂ without emitter is not
1199 possible due to an unacceptably high drip loss.

1200

1201 Trindade and others (2013) evaluated the use of a combined oxygen scavenger and carbon
1202 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
1204 emitter applied in vacuum-packages. The percentage level of CO₂ generated in the vacuum-
1205 packages including an O₂ scavenger/CO₂ emitter was reported to be 45% CO₂ after about 17
1206 days of storage. The same CO₂ level was measured in the control vacuum-packages without
1207 emitter, speculated by the authors to be a result of CO₂ emission by anaerobic
1208 microorganisms. The CO₂ level is unexpectedly low for vacuum-packages with O₂
1209 scavenger/CO₂ emitter, considering the limited available volume and that the O₂ 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
1214 with a CO₂-releasing packaging structure (CSP Technologies, technology unknown) was
1215 found to be effective at controlling the proliferation of artificially inoculated *Listeria*
1216 *monocytogenes*, as well as TVC and Enterobacteriaceae on a vacuum-packaged ready-to-eat
1217 meat product (cooked ham) stored at different temperatures and monitored over a 4-week
1218 period. The percentage levels of CO₂ 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
1220 of *L. monocytogenes* is not an industrially relevant scenario as the focus is on preventing
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
1223 of documented sampling data (bacterial counts) during the storage time. The 2 latter studies
1224 are not included in Table 6 as the underlying technologies (active substances) of the emitters
1225 have not been published.

1226

1227 Comparisons of different CO₂ emitter concepts are challenging. Differences in pre-handling
1228 of the food product, storage conditions and temperature, type of packaging material, g/p
1229 ratio, package size, and gas composition are all variables that will have an impact on the
1230 effect that the CO₂ emitter contributes to the shelf life of the food product. Another
1231 important factor concerning the evaluation of CO₂ emitter that is rarely documented is the
1232 material and density of the emitter substrate. The type and structure of the substrate in
1233 which the active ingredients are incorporated is of importance for liquid absorption and the
1234 amount and rate of CO₂ release. Examples of materials applied in different layers of CO₂
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
1237 ingredients may be evenly distributed in pores of the substrate material or in a bulk deposit
1238 in the core of the substrate. These aspects should be taken into consideration when
1239 comparing the performance of different emitter systems.

1240

1241 The results of earlier scientific studies, as summarized in the previous paragraphs, have
1242 shown that an optimal effect of CO₂ emitters can only be achieved when the emitter
1243 capacity is optimized, that is adapted to the physiological properties and weight of the food
1244 product in question. An emitter with an optimal capacity will ensure an adequate CO₂ level,
1245 counteract formation of negative pressure within the package, ensure sufficient liquid
1246 absorption, and extend shelf life. Optimization of the emitter capacity was investigated in a
1247 study by Hansen and others (2009b) for salmon fillets in MA-packages with different fillet
1248 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 CO₂ emitter technologies more
1252 flexible and suitable for a broader range of food products.

1253

1254 With regard to commercialization, there are different emitters already on the market today.

1255 Emitters based on sodium bicarbonate and citric acid include CO₂ Freshpads (CO₂

1256 Technologies, Urbandale, Iowa, USA)(Kerry 2014), SuperFresh (Vartdal Plastindustri AS,

1257 Vartdal, Norway), and the Active CO₂ pad (CellComb AB, Säffle, Sweden). In addition,

1258 UltraZap® XtendaPak (Paper Pak Industries, La Verne, CA, USA), and the CO₂Pad (McAirlaid's

1259 GmbH, Steinfurt, Germany) are based on other CO₂-releasing concepts.

1260

1261 **Antimicrobial Packaging Systems**

1262 Antimicrobial food packaging presents a system designed to inhibit the growth of spoilage

1263 and pathogenic microorganisms. In this review, the most studied antimicrobial food

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);

1266 and organic acids, their derivatives and other organic compounds (Table 10). Furthermore,

1267 antimicrobial nanoparticles are reviewed separately as the nano-size itself either increases

1268 or enables the antimicrobial activity (Table 11).

1269

1270 **Essential Oils (EOs)**

1271 Recent interest in reducing the use of petroleum-based additives as active materials for food
1272 preservation has led to the application of natural additives both for the benefit of the
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
1275 possess strong antimicrobial properties. In addition, most of them are classified as GRAS
1276 (Ruiz-Navajas and others 2013) and, as a result, EOs have been extensively studied as
1277 additives in bio-based emulsified films and coatings. Many scientific publications are
1278 connected to the potential interest in this type of active packaging but without any real
1279 application to food. Some studies, however, have demonstrated the effectiveness of EO-
1280 enriched packages containing food and these are presented in Table 7.

1281

1282 Cinnamon essential oil (CEO) is among the most studied EOs in active materials. Gherardi
1283 and others (2016) showed that a multilayer material containing about 18 and 10% of
1284 cinnamaldehyde as the major compound of the selected EO showed high activity against *E.*
1285 *coli* O157:H7 and *S. cerevisiae*, as the material reduced both microorganisms by 3 log
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
1288 avoid too much loss of volatile substances, an antimicrobial packaging material was
1289 developed by incorporating a cinnamon essential oil/ β -cyclodextrin inclusion complex into
1290 polylactic acid nanofibers via an electrospinning technique (Wen and others 2016).

1291 Application in the preservation of pork (25 °C) showed that the sample packed with the
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 10^3 – 10^4 CFU/g on

1294 the first day and the unpacked control pork reached an excessive number of colonies after 4
1295 days (above 1.10^7 CFU/g). Another option to control the release of major compounds of CEO
1296 is to reversibly anchor cinnamaldehyde to a polymer, such as chitosan films, via imino-
1297 covalent bonding (Higuera and others 2015). The antimicrobial properties of chitosan-Schiff
1298 base films in milk inoculated with *Listeria monocytogenes* led to a growth inhibition for 12
1299 days under refrigeration conditions.

1300

1301 Carvacrol is another EO regularly used as a bio-based bioactive compound. However, the
1302 synergistic antimicrobial effect of different EOs on food has been less frequently studied.
1303 One example is the study by Campos-Requena and others (2015) based on carvacrol and
1304 thymol, both included in HDPE/modified montmorillonite nanocomposite films. A synergistic
1305 antimicrobial effect was observed with *Botrytis cinerea*, when the films were applied
1306 through indirect contact with strawberries. The half maximal inhibitory concentration (IC₅₀)
1307 of the EOs in the film was reduced from 40.4 mg/g (carvacrol only) to 13.2 mg/g (both EOs
1308 50:50). Knowing that the major volatile compounds of oregano EO is carvacrol, Rodriguez-
1309 Garcia and others (2016) evaluated the effect of oregano EO applied within pectin coatings
1310 on the inhibition of *Alternaria alternata* on tomatoes. The authors showed that 25.9 g/L was
1311 effective in inhibiting microbial growth.

1312

1313 To enhance safety and shelf life of cooked cured ham, Ruiz-Navajas and others (2015)
1314 studied 2 Spanish endemic species of thyme, *Thymus piperella* and *Thymus moroderi*. They
1315 reported that *T. piperella* had a higher effect than *T. moroderi*, probably due to the higher
1316 concentration of carvacrol in the former (predominant compound, 31,9%). Addition of both

1317 EOs into films (from 1 to 2%) significantly decreased the aerobic mesophilic and lactic acid
1318 bacteria counts in food samples, with lowest counts for *T. piperella* at 2%. After 21 days, for
1319 example, the addition of 2% EOs led to a reduction of 0.87 and 0.53 log cycles of aerobic
1320 mesophilic bacteria compared to the uncoated samples of *T. piperella*- and *T. moroderi*-
1321 based films, respectively. In another study with thyme EOs, Quesada and others (2016)
1322 designed an active packaging system for the shelf-life extension of sliced ready-to-eat
1323 cooked pork during refrigerated storage. Interestingly, the package included an inner surface
1324 coated with a chitosan film with thyme essential oil (0%, 0.5%, 1%, and 2%) and was not in
1325 direct contact with the meat to avoid modification of organoleptic properties. The authors
1326 reported that yeast populations were affected by the presence of thyme EO and the yeast
1327 counts decreased as a function of the EO dose in the film, especially during the first 21 days
1328 of storage.

1329

1330 Arfat and others (2015) investigated microbiological and sensory changes of sea bass slices
1331 wrapped with fish protein/fish gelatin composite films incorporated with basil leaf essential
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
1334 incorporating BEOs (10-12 days) compared to the control (6 days). *Allium spp.* extract and
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%
1337 Proallium®). With the latter, Lee and others (2016) demonstrated that crab sticks packed
1338 with starfish gelatin films containing 0.05% vanillin exhibited antimicrobial activity against *L.*
1339 *monocytogenes* previously inoculated on the food product.

1340

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

1361 Incorporation of proteins, particularly enzymes and bacteriocins, into food packaging to control
1362 spoilage caused by food pathogenic microorganisms has been an area of research for several
1363 decades (Table 8).

1364 Enzymes can serve as effective antimicrobials in food packaging by being chemically bonded to,
1365 or physically entrapped in, packaging films. As an antimicrobial enzyme, lysozyme can destroy
1366 the glycosidic bonds of the Gram-positive bacterial peptidoglycans. Lysozyme incorporated into
1367 whey protein films (204 mg/g of film) migrated into the food and inhibited the growth of
1368 *Listeria monocytogenes* to 4.4 log CFU/cm², extending the shelf life of smoked salmon (Min and
1369 others 2005). Barbiroli and others (2012) reported incorporation of lysozyme and lactoferrin
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
1373 preventing growth of aerobic bacteria in the meat sample, giving almost 1 log cycle reduction
1374 with respect to the control. Lysozyme is accepted by the U.S. Food and Drug Administration
1375 (FDA 2001) as an antimicrobial agent in casings for frankfurters, and in Europe the use of
1376 lysozyme (E1105) falls under Directive 95/2/EC on food additives (European Union 1995).

1377

1378 Bacteriocins are peptides or small proteins, produced by some species of lactic acid bacteria
1379 (LAB), which inhibit the growth of food spoilage bacteria, mainly Gram-positive bacteria. The
1380 bacteriocin nisin has been successfully incorporated into methylcellulose/hydroxypropyl
1381 methylcellulose coatings (Franklin and others 2004) or PE films (Siragusa and others 1999), and

1382 it has been coated on LDPE films (Mauriello and others 2005; Neetoo and others 2008) or
1383 paperboard (Lee and others 2004). Subsequently, the effective inhibition of bacterial growth
1384 was achieved in such foods as hot dogs, beef, milk, cold-smoked salmon, and orange juice. For
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
1387 surface of hot dogs by greater than 2 log CFU per package after 60 days of refrigerated storage
1388 (Franklin and others 2004). Similarly, it was established that nisin-coated LDPE films were
1389 effective in inhibiting the bacterial flora in milk stored at 4 °C for 7 days, and the most
1390 significant results were observed in raw milk and pasteurized milk with a reduction of 0.9 and
1391 1.3 log, respectively. Nisin (E234) has been authorized for food preservation in Europe under
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
1395 films or polyamide coatings effectively inhibited *Brochothrix thermosphacta*, coliform bacteria
1396 growth and extended the shelf life of beef (Cutter and others 2001; Kim and others 2002) and
1397 fresh oysters (Kim and others 2002). According to the research of Khan and others (2016), the
1398 immobilization of nisin and EDTA on the surface of the cellulose nanocrystal/chitosan-films, by
1399 using genipin as a cross-linking agent, restricted the growth of psychrotrophs, mesophiles and
1400 *Lactobacillus* spp. in fresh pork loin meats, and increased the microbiological shelf life of the
1401 meat sample by more than 5 weeks. The films also reduced the counts of *E. coli* and *L.*
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

1404 sorbitan monooleate-coatings on polymeric films of different hydrophobicity (polyvinylchloride,
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
1410 others 2007), lactocins (Massani and others 2014), natamycin (De Oliveira and others 2007),
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*,
1413 *Lactobacillus plantarum*, *Listeria innocua*, *Salmonella* spp.) or fungal (*Penicillium roqueforti*)
1414 growth on cooked ham, Wieners (2.5 log reduction), Gorgonzola cheese, and sliced ham (0.5-2
1415 log reduction), respectively.

1416

1417 Further research effort is still needed to evaluate the release of enzymes and bacteriocins from
1418 various films and coatings into packaging, as well as diffusion to the surface of the food.

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

1423 exploitation. Nevertheless, the use of enzymes and bacteriocins in combination with other

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 ϵ -polylysine are inherently antimicrobial and are used in films
1429 and coatings (Table 9). ϵ -Polylysine is a natural antimicrobial polypeptide that is effective
1430 against Gram-positive and Gram-negative bacteria. However, only a few studies have reported
1431 on polylysine incorporation into packaging materials. For example, Zinoviadou and others
1432 (2010) developed ϵ -polylysine-containing whey protein films that significantly reduced the
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
1440 others 2010) and bio-based polymers such as carboxymethylcellulose (Youssef and others 2016)
1441 or used as a coating on plastic films (Joerger and others 2009). When chitosan-incorporated
1442 LDPE films were applied on fresh sliced red meats, microorganisms on the meat surface were
1443 not inhibited but significant extension of red color shelf life was observed in refrigerated
1444 samples (Park and others 2010). Meanwhile, bio-nanocomposite films containing chitosan had
1445 an effect on the total bacterial counts, mold and yeast counts, and coliforms in soft white
1446 cheese during 30 days of storage at 7 °C, and increased its shelf life (Youssef and others 2016).
1447 Total bacterial counts significantly decreased during storage especially for samples that were

1448 coated with preservative films. Furthermore, the coliform, mold and yeast organisms in soft
1449 cheese were inhibited by the active films. Moreover, ethylene copolymer film was coated with
1450 chitosan through attachment of the polymer to the corona-treated surface of the film, and the
1451 antimicrobial activity of the composite film against *Listeria monocytogenes* Scott A was tested
1452 on turkey breast and a log reduction of about 1.7 after 10 days and 1.2 after 15 days at 4 °C was
1453 achieved (Joerger and others 2009).

1454

1455 Ye and others (2008a) determined that chitosan-coated plastic films were not able to control
1456 the growth of *L. monocytogenes* on ham steaks and, therefore, evaluated the antilisterial
1457 efficacy of chitosan-coated plastic films incorporating 5 additional GRAS antimicrobials: nisin,
1458 sodium lactate, sodium acetate, potassium sorbate, and sodium benzoate. The incorporation of
1459 those antimicrobials into chitosan-coated plastic film retarded or inhibited the growth of *L.*
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*
1462 being slightly lower than the initial inoculum. Similarly, the same films inhibited the growth of *L.*
1463 *monocytogenes* on cold-smoked salmon samples for at least 6 weeks (Ye and others 2008b).
1464 However, the authors indicated that sensory studies are needed before this technology is
1465 further developed (Ye and others 2008a).

1466

1467 The antimicrobial activity of chitosan (low molecular weight, 150 kDa, 75-85% deacetylation)
1468 coating with 5-10% of lauric arginate ester (LAE), 2-20% of sodium lactate, and 0,3-0,6% of
1469 sorbic acid (alone or in combination) on PLA films was verified using *Listeria innocua* and

1470 *Salmonella typhimurium* (Guo and others 2014). Most effective combinations were 5%
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/cm² for both
1475 films. The films also reduced the growth of *L. monocytogenes* on the surface of ready-to-eat
1476 meat by 2.5-3 log CFU/cm² during storage of 3 or 5 weeks at 10 °C. For *S. typhimurium* the
1477 reduction was 1.5 log CFU/cm².

1478

1479 The effect of chitosan used in combination with nisin, potassium sorbate, or silver-substituted
1480 zeolite incorporated into LDPE on the physicochemical and microbial quality of chicken
1481 drumsticks stored at 5 °C for 6 days was also investigated (Soysal and others 2015). Total
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,
1484 respectively, than those of samples packed in control bags. Moreover, samples packed in active
1485 bags had lower TBARS values than those of samples in control bags. The exploitation of GRAS
1486 antimicrobials nisin and potassium sorbate in food packaging is straightforward, whereas the
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
1489 the provisional list for use in accordance with national law. In another study, chitosan films
1490 were developed by incorporating lauric arginate ester (LAE) and their antimicrobial activity
1491 against mesophiles, psychrophiles, *Pseudomonas spp.*, coliforms, lactic acid bacteria, hydrogen

1492 sulfide-producing bacteria, yeast and fungi was evaluated on chicken breast fillets at 2, 6, and 8
1493 days (Higuera and others 2013). Chitosan films demonstrated antimicrobial activity in the
1494 range of 0.47-2.96 log reduction, dependent on time and bacterial group studied, while the
1495 incorporation of 5% LAE in the film increased antimicrobial activity to 1.78-5.81 log reduction.

1496
1497 It should be noted that chitosan has been given GRAS status by the U.S. FDA (FDA 2002, 2005,
1498 2011) for agricultural and medicinal purposes, but it is not yet specifically approved as an
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
1501 antimicrobial properties, packaging coatings and films prepared from such biopolymers exhibit
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
1506 sensory properties of food as well as to validate already developed packaging by using
1507 commercial food products held under real storage conditions.

1508

1509 **Organic Acids, their Derivatives and other Organic Compounds**

1510 Some organic compounds such as selected organic acids and their derivatives, exhibit
1511 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
1516 increased in all the samples, less growth was observed in minced beef packed with active films
1517 compared to the control samples at the end of a 10-day evaluation period. The authors
1518 reported a reduction in total bacteria counts of approximately 1 log CFU/g. The results
1519 identified the potential of active films containing citric acid to extend the shelf life of minced
1520 beef. However, further research needs to be conducted to improve the limited antimicrobial
1521 effect demonstrated in this study.

1522
1523 **Sorbic Acid.** García-Soto and others (2015) incorporated 0.5% and 1% of sorbic acid and 8% of
1524 algal extract (*Fucus spiralis*) into PLA films to protect the flat fish megrim (*Lepidorhombus*
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
1527 lower mean values for aerobes and Enterobacteriaceae after 7 days of storage. Although the
1528 results obtained do not demonstrate a significant antimicrobial effect at the end of the shelf life
1529 (11 days), improved sensory properties (external odor, gill appearance, and odor) were
1530 reported for megrim packed with active films, while control samples were considered
1531 unacceptable from a sensorial point of view. Limjaroen and others (2005) incorporated sorbic
1532 acid into solvent cast poly(vinylidene chloride) (PVDC) films. Beef bologna and cheddar cheese,
1533 inoculated with *L. monocytogenes* (10^3 and 10^5 CFU/g each), were wrapped in PVDC films
1534 containing 1.5 or 3% w/v sorbic acid. After 28 days of storage at 4 °C, lower *L. monocytogenes*

1535 counts were obtained in beef bologna samples packed with active films and inoculated with 10^5
1536 CFU/g (4.4 log lower for both sorbic acid films, compared to the control). In the inoculated
1537 cheddar cheese samples, the active films did neither significantly affected the growth of the
1538 inoculated *L. monocytogenes* nor that of mesophilic aerobic bacteria after 35 days of storage at
1539 4 °C. Beef samples inoculated with 10^3 CFU/g, in contrast, demonstrated 6.5 and 7.2 log lower
1540 *L. monocytogenes* counts for 1.5 and 3% sorbic acid films, respectively, compared to the
1541 control. Moreover, mesophilic aerobic bacteria and LAB counts in the beef packages with the
1542 active films were found to be around 4 and 6 log lower than in control samples with initial *L.*
1543 *monocytogenes* inoculums of 10^5 and 10^3 CFU/g, respectively. This research, however, has
1544 some drawbacks, especially because the use of sorbic acid as an additive in meat products is
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
1548 of meat products at the maximum level of 1 g/kg or *quantum satis*. Moreover, the reported
1549 sample preparation, a solution-casting, laboratory-scale method, cannot be applied on an
1550 industrial scale. This trial should be repeated using melt (extrusion), however, the high
1551 temperature may influence the sorbic acid activity.

1552

1553 **Potassium sorbate.** Cestari and others (2015) developed thermoplastic starch/PBAT blended
1554 films with 5% potassium sorbate content to prevent microbial growth in restructured chicken
1555 steaks during frozen storage. After 30 days of storage, *Escherichia coli* (initial count 1.94 log
1556 CFU/g) was not detected in samples packed with potassium sorbate films, while *E. coli* was

1557 detected in control samples (1.3 log CFU/g). In chicken steaks packed with the active films, *E.*
1558 *coli* was kept under the detection limit until completion of frozen storage (150 days). Kaya and
1559 others (2015) reported the use of potassium sorbate and/or sodium lactate (3% applied alone
1560 and 1.5% of each, applied in combination) in brine to protect smoked rainbow trout
1561 (*Oncorhynchus mykiss*) fillets against microbial growth. The trout fillets were kept in 8% NaCl
1562 brine for 12 h before smoking. After 4 weeks of storage at 6 ± 1 °C, total aerobic mesophilic
1563 bacteria counts were shown to be about 3, 2.1 and 1.7 log CFU/g lower for trout kept in
1564 potassium sorbate, sodium lactate and its combination, respectively, compared to the control
1565 (about 8.2 CFU/g) kept in brine without preservatives. Similar results were observed for yeast
1566 and molds. Additionally, after 5 weeks of storage an identification of the bacteria species in the
1567 applied samples was performed. With the exception of the trout kept in potassium sorbate
1568 brine, *Serratia liquefaciens*, which is considered as one of the main pathogens and spoilage
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

1572 **Potassium metabisulfite.** Several fruits and vegetables are highly susceptible to enzymatic
1573 changes, and the application of some antimicrobials can provide additional properties against
1574 this problem. Foralosso and others (2014) tested PVC films that contained a 0.1, 1, or 2% w/w
1575 mixture of pure and encapsulated potassium metabisulfite (ratio 1:1) as an active
1576 (antimicrobial, antioxidant and antibrowning) substance. Cut Gala apples (*Malus domestica*)
1577 were wrapped in the active PVC films, and stored at 4, 8, 12, 16, and 20 °C and 30% RH.
1578 Samples wrapped in PVC films with 1 and 2% potassium metabisulfite mixtures resulted in a

1579 lower browning index which was rated to be around 60 and 50%, respectively, compared to the
1580 control (around 90%), and a shelf-life extension from 4 to 8 days for apples stored at 8 °C was
1581 reported. Samples wrapped using 2% potassium metabisulfite mixtures and stored at 4 °C
1582 demonstrated toxicological and microbiological stability (migration of sulfites below 10 mg/kg
1583 SO₂, according to Brazilian regulation for plastic materials in contact with food; and microbial
1584 counts below 10⁶ CFU/g, considered as the quality threshold by the authors) throughout the 20-
1585 day storage period. The active film provided the conditions suitable for apple consumption up
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
1590 cellulose micro-particles (4% w/w) in poly(ε-caprolactone (PCL) films and evaluated their
1591 antibacterial activity on packed sliced salami inoculated with *L. monocytogenes* (10⁴ CFU/g).
1592 After 14 days of storage at 4 °C in contact with the active PCL films, 50% of total colony-forming
1593 units (about 8 log CFU/g) of *L. monocytogenes* did not survive. The packaging also led to a
1594 decrease in the growth of *E. coli* and *S. aureus*. Moreover, active films containing 4% of oxidized
1595 regenerated cellulose micro-particles reduced the oxygen and water permeability by 93 and
1596 70%, respectively.

1597
1598 **Allyl isothiocyanate.** A high antibacterial activity is reported for allyl isothiocyanate (AITC)
1599 against a wide range of bacteria (Kim and others 2015). Pang and others (2013) reported the
1600 positive effect of using AITC (18 and 36 µg/L) in the vapor phase when applied, alone and in

1601 combination with MAP (49% CO₂/0.5% O₂/50.5% N₂), to catfish fillets stored at different
1602 temperatures. The authors observed that AITC (alone or in combination with MAP) had an
1603 antimicrobial effect against *Pseudomonas aeruginosa* and extended the shelf life of fresh
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*
1606 counts at a level of about 3 CFU/g during 23 days, compared to the control without MAP (about
1607 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
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
1610 at the end of storage. However, due to the pungency and strong smell of AITC, a sensory
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
1615 Commercial packaging solutions containing AITC can be found in a variety of formats (sheets,
1616 labels and films) on the Japanese market under the trademark Wasaouro™ (Mitsubishi-Kagaku
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
1620 countries can differ and data regarding current status can change (such as the approval status
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

1623 uses as a food additive. Therein, it is stated that AITC is “an efficient alternative to already
1624 approved preservation techniques for a range of foods,” such as bakery products (including all
1625 types of pre-packed bread and fine bakery ware), all types of cheese, fruits, and vegetables
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
1628 Panel 2015). The main hurdle to commercialization of active packaging solutions containing
1629 organic compounds are regulatory requirements. Therefore, research efforts should be focused
1630 on the development of tailor-made active packaging solutions that comply with the specific
1631 legislation for each food product.

1632

1633 **Nanoparticles**

1634 Antimicrobial nanomaterials represent an increasingly important component of some active
1635 packaging for food applications (Ayhan 2013). Antimicrobial nanoparticles (particles between 1-
1636 100 nm in size) are incorporated into a polymer matrix with the aim of prolonging the shelf life
1637 of packaged food. High surface-to-volume ratio and enhanced surface reactivity of the nano-
1638 sized antimicrobial agents cause inactivation of microorganisms more effectively than their
1639 micro or macro-scale counterparts (Radusin and others 2016). The preparation of food
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
1651 nanoparticles into different polymer matrices, in combination with other additives or
1652 nanoparticles, can significantly prolong the shelf life of different foodstuffs. Li and others (2017)
1653 reported that rice stored in LDPE without Ag/TiO₂ showed a serious mildew condition after one
1654 month with increased total plate counts (TPC) from 4.84 to 7.15 log cfu/g, while the rice stored
1655 in a nanocomposite based on LDPE with Ag/TiO₂ had a low TPC of 5.48 log cfu/g. Mihaly
1656 Cozmuta and others (2015) reported that the microbiological safety of bread stored in Ag/TiO₂-
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
1659 compared to the bread stored in an open atmosphere or in a commonly used plastic packaging.
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
1662 resulted in lower lipid oxidation of chicken breast fillets, while Panea and others (2014)
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

1667 effective in prolonging the shelf life of orange juice (up to 28 days). ZnO has also been used as
1668 an antimicrobial agent added to active packaging films for packaging fresh poultry meat by
1669 Akbar and Anal (2014), and they showed a reduction of the initial bacterial counts (*S. aureus*
1670 and *S. typhimurium*) by 2 log within 24h of incubation at 8 ± 1 °C. After 6 days there were no
1671 viable cells of *S. aureus*, and no *S. typhimurium* after 8 days of incubation.

1672

1673 Titanium dioxide (TiO₂) has been studied as antimicrobial nanoparticles in LDPE for the
1674 packaging of fresh pears, and a decrease in mesophilic bacteria from 3.14 to less than 2 log
1675 CFU/g for the entire storage period (17 days) was recorded, whereas for neat LDPE cell loads
1676 increased from 3.19 to 4.02 log CFU/g. Furthermore, yeasts decreased from 2.45 to less than 2
1677 log CFU/g, whereas those for the control sample increased from 2.1 to 3.37 log CFU/g (Bodaghi
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

1683 As reported in the previous sections, the use of antimicrobial nanoparticles has great potential
1684 in preserving the microbial quality of the food systems. In this context, the appropriate
1685 antimicrobial agent needs to be selected according to the targeted food. Additionally, the
1686 impact of nanoparticles on the properties of the packaging films, such as barrier properties and
1687 transparency, should also be considered. However, the safety evaluation and approval for use
1688 of such nanoparticles in food packaging remains the greatest challenge due to the difficulties in

1689 the evaluation of the safety of nanoparticles in general (Radusin and others 2016) as well as
1690 constraints associated with the current legislative landscape (Amenta and others 2015, Radusin
1691 and others 2016, Rauscher and others 2017).

1692

1693 Over the last decade, various studies have been conducted in this area and several scientific
1694 reviews have been published. However, these have mostly focused on technology and several
1695 mechanisms, as well as “*in vitro*” studies on culture media. There has been little research
1696 involving real food packaging systems. Such research, however, is of great importance, since
1697 the antimicrobial activity of the active agents with culture media does not necessarily correlate
1698 with the antimicrobial activity in the food. This is mainly due to the complex structure of the
1699 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
1702 have to be considered. Firstly, the food system has to be fully understood in terms of its
1703 components, and physical and chemical characteristics, such as pH, and water activity, as well
1704 as its microbiological aspects, including identification of those microorganisms that are
1705 desirable and undesirable. A suitable antimicrobial active agent should be selected with respect
1706 to all these characteristics. In particular, the antimicrobial spectrum and the efficiency of the
1707 agent should target the microorganisms that limit the shelf life of the particular food. According
1708 to the international standard on the measurement of antibacterial activity of plastics and other
1709 non-porous surfaces (ISO 22196 2011), derived from Japanese Industrial Standard (JIS Z 2801
1710 2000), a decrease of the number of microorganisms in the magnitude of 2 log colony forming

1711 units (CFU)/cm² is required to demonstrate antimicrobial efficacy. In food systems, shelf-life
1712 tests have to be performed to evaluate the efficiency of the antimicrobial film for the selected
1713 product. In this context, the maximum permitted level of microorganisms in a food is very
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
1716 be marketed. In the EU, for instance, the microbiological criteria for foodstuffs are regulated by
1717 the Commission Regulation (EC) No 2073/2005) (European Commission 2005). For some food
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,
1720 the influence of the food on the efficiency of the antimicrobial agent should be considered
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

1724 A second consideration is the storage conditions of the packed food since the temperature or
1725 relative humidity may affect the release and/or the efficiency of the active agent. A third factor
1726 involves selection of antimicrobial agents that do not cause any undesired changes in the food,
1727 such as the sensory properties. The last aspect to consider is that the addition of antimicrobial
1728 agents should not result in undesirable changes in the packaging material, such as barrier,
1729 sealing and adhesion properties, transparency, or glossiness, and it should not cause any
1730 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
1739 highly product-specific. Thus, to achieve an optimal activity or capacity of the desired active
1740 packaging system, product-tailored concepts have to be applied. Thereby, it is crucial to
1741 consider all the influencing factors, such as the physical/chemical/physiological properties of
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
1750 scientists with a better understanding of the potential and the benefits of active packaging
1751 technologies and, hence, assist in accelerating their commercial adoption.

1752

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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

1768 Zehra Ayhan: Ethylene absorbers

1769 Ramune Rutkaite: Antioxidant releasers; Antimicrobial packaging systems (enzymes and
1770 bacteriocins, polymers)

1771 Tanja Radusin: Antimicrobial packaging systems (nanoparticles)

1772 Patrycja Suminska: Moisture scavengers; Antimicrobial packaging systems (organic acids, their
1773 derivatives and other organic compounds)

1774 Begonya Marcos: Antioxidant releasers, Moisture scavengers, Antimicrobial packaging systems
1775 (organic acids, their derivatives and other organic compounds)

1776 Veronique Coma: Antimicrobial packaging systems (essential oils)

1777

1778

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1779

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2591 **Tables**

2592 See separate file

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Table 1: Potential active packaging for food applications.

Type of Active Packaging	Type of Food	Potential Benefit
Active scavenging systems (absorber)		
Oxygen scavenger	(Sliced) cooked meat products Grated cheese, (par-baked) bakery products Fruit and vegetable juices Seeds, nuts, and oils; fat-containing instant powders, fried snacks; dried meat products	Prevention of discolouration Prevention of mold growth Retention of vitamin C content, prevention of browning Prevention of rancidity
Moisture scavenger	Mushrooms, tomatoes, strawberries, maize, grains, seeds, fresh fish, and meat	Extension of shelf life through maintaining moisture content, decrease in moisture condensation in the packaging, positive impact on the appearance, reduction in browning or discoloration
Ethylene absorber	Climacteric fruits and vegetables	Reduction in ripening and senescence, thereby enhancing quality and prolonging shelf-life
Active releasing systems (emitter)		
Antioxidant releaser	Fresh fatty fish and meat; fat-containing instant powders; seeds, nuts, and oils; fried products Fresh fish and meat	Improvement of oxidative stability Extension of microbiological shelf life, reduction in head space volume of modified atmosphere packaging
Carbon dioxide emitter	Fresh and processed meat, fresh and smoked fish, fresh seafood, dairy products, fresh and processed fruits and vegetables, grain, cereals and bakery products, ready-to-eat meals	Inhibition or retardation of bacterial growth, extension of the shelf-life

Table 2: Oxygen-scavenging food packaging systems

Active Substances	Matrix / Packaging Application	Food Application	Benefit	Reference
Iron (Fe)	Multilayer-container: PP/EVOH/OS/PP	Meatballs	Significant inhibition of lipid oxidation and prevention of flavor and color change during up to 9 months of storage	Shin and others (2009)
	Incorporated into laminate: PET/Alu/OS*/PE *ABSO ₂ RB®	Hot-filled cheese spread	1.5 times higher Vitamin C content, maintenance of product quality (physiochemical & organoleptic) after 1 year	Gomes and others (2009)
	Mixture of Fe nanoparticles, activated carbon, NaCl and CaCl ₂ in sachets	Roasted sunflower seeds and walnuts	Inhibition of lipid oxidation: 2-3 times lower PV and half of AnV after 120 days	Mu and others (2013)
Ascorbic acid	Multilayer film: PET/Alu/PE/OS*/PE *SHELPLUS™ O ₂ 2400	Salami bread	Compensation of pinhole defects (10 µm) of packaging: Reduced color change (ΔE -22%) and more than 4 times reduced lipid oxidation after 300 days	Sängerlaub and others (2013)
	LLDPE-films containing ascorbic acid and zinc or iron-powders	Bun and bread slices	Overall shelf-life extension: Retarded microbial growth from 2 to 5 days, prolonged sensorial acceptance from 2 to 5-6 days	Mathe and others (2011)
Photosensitive dyes	Photosensitive dyes (eosin & curcumin) and synthesized PFO incorporated in ethyl cellulose polymer cast films	Sunflower oil	Improvement of oxidative stability: Up to 5 times lower PV and hexanal concentration after 12 weeks	Maloba and others (1996)
	EVOH / OS*/CPP laminate *Photosensitive dye and reducible organic compound	Orange juice	Retention of vitamin C content (3.8 times at 25 °C, 1.4 times at 4 °C), prevention of non-enzymatic browning at 4 °C and 23% reduction at 25 °C; after 1 year.	Zerdin and others (2003)
	Commercial barrier packaging (Nupak™) lined with OS-sheet: EVOH/OS*/EVA *Zero ₂ ™	Probiotic yogurt	Improvement of the viability of probiotic bacteria by removing ~90% of the initial dissolved O ₂ after the first day	Miller and others (2003)
	Zero ₂ ™ OS-film laminate: OPET/EVOH/OS/CPP	Milk	Prevention of stale flavor development: 23-41% fewer stale flavor volatiles after 14 weeks	Perkins and others (2007)
Unsaturated hydrocarbon dienes	OS* incorporated in PET-bottles *AMOSORB	Citrus juice model system	50% Vitamin C retention at 35 °C and up to 75% at 4 °C; and browning prevention. After 16 weeks.	Baiano and others (2004)
	OS*-PET film, cast extruded *AMOSORB	Fresh-cut banana	Reduction in oxidation (browning): ~50% less color difference (ΔE) after 3 days	Galdi and Incarnato (2011)
Palladium (Pd) (+ hydrogen)	Pd-deposited film: PET/SiO _x /Pd	Cooked cured sliced ham	Prevention of discoloration (redness) for 21 days of storage under illumination	Yildirim and others (2015); Hutter and others (2016); Röcker and others (2016)
		Bakery products: partially baked buns, toast bread slices and gluten-free bread slices	Retard of mould growth from 2 to 8-10 days	Rüegg and others (2016)

Table 3: Moisture-scavenging food packaging systems.

Active Substances	Matrix / Packaging Application	Food Application	Benefit	Reference
Bentonite/sorbitol/calcium chloride	Powder in trays/bags in the package	Mushrooms	Decrease in moisture condensation inside the package; better product appearance: lower browning index (BI 14.8) compared to control (BI 18); extension of shelf life from 1-3 days to 5 days at 10 °C	Mahajan and others (2008)
Poly(acrylic acid) sodium salt	Powder in porous "tea bag" in sealed containers	Maize	Reduction in aflatoxin contamination to below European aflatoxin contamination B1 limits of 4 ng/g grain	Mbuge and others (2016)
Sodium chloride	Thermoformed multilayer trays: PE/foamed hygroscopic ionomer-NaCl/PE	Tomatoes and strawberries	Regulation of in-package RH below 97% during 7 days at different temperatures	Rux and others (2016)
	Thermoformed multilayer trays: PP/foamed and stretched PP - NaCl/EVOH/PE	Mushrooms	Regulation of in-package RH at 93% during 6 days at 7 °C, better color appearance, gill exposure and less incidence of decay after 6 days	Rux and others (2015)
		Mushrooms	Decrease in water loss from 4.5 to 1.3 g at 5 °C	Singh and others (2010a,b)

Table 4: Ethylene-absorbing food packaging systems.

Active Substances	Matrix / Packaging Application	Food Application	Benefit	Reference
Charcoal w/ palladium chloride	Absorbent (paper packets) in metal tray with glass cover	Fresh-cut kiwifruit, banana, spinach	Reduction in softening of fresh-cut kiwifruit and bananas, and chlorophyll loss in spinach leaves, no accumulation of ethylene in the trays for kiwifruit slices, banana sections, broccoli and spinach for 3 days at 20 °C	Abe and Watada (1991)
Nano-Ag, Nano-TiO₂ and kaolin	PE film	Strawberry	Quality improvement: sensory, physicochemical and physiological properties (decay rate, anthocyanin, and malondialdehyde contents were decreased to 16.7%, 26.3 mg/100g, 66.3 μmol/g for nano-packing and 26.8%, 31.9 mg/100g, 75.4 μmol/g for normal packing, respectively)	Yang and others (2010)
	PE film	Chinese bayberry	Controlling green mold decay, reduced respiration rate and ethylene production (49.6% and 25.9%, respectively, for combined treatment of hot air and nanopackaging which was lower than the control) and providing firmer fruit for 8 days at 1 °C	Wang and others (2010)
Nano-Ag, Nano-TiO₂ and montmorillonite	PE film	Chinese jujube	Positive effects on physicochemical and sensory quality, prevention of fruit softening, weight loss, browning and climactic evolution, and ethylene control (maximum ethylene content of 17.6 μL/kg h for the control on 3 rd day and 9.2 μL/kg h for nanopackaging on the 6 th day of storage)	Li and others (2009)
	PE film	Kiwifruit	Inhibition of ethylene production (57.4% lower headspace ethylene concentration in nanopackaging), prevention of physiological changes, delay in ripening	Hu and others (2011)
Nano-ZnO	PVC film coated with nano ZnO	Fresh-cut apple	Reduction in fruit decay rate (21.5% for nano-coated PVC, 42.4% for uncoated PVC on 12 th day at 4 °C), slowdown in ethylene production (maximum ethylene content 40 μL/kg day for nanopackaging on 9 th day and 70 μL/kg day for the control on 6 th day storage), maintenance of °Brix and titratable acidity and inhibition of enzyme activity	Li and others (2011)
Zeolite-based various aluminosilicate minerals	LDPE films	Broccoli florets	Improvement of overall quality and increase in shelf-life up to 20 days at 4 °C	Esturk and others (2014)

Zeolite-based impregnated with $KMnO_4$	HDPE films	Kiwifruit	Firmer texture, higher vitamin C content, no shelf life provided	Küçük (2006)
Zeolite fine particles of mordenite framework inverted-type zeolite	LDPE film	Mango	Extension of shelf-life up to 40 days at 12 °C, reduction in weight loss, maintaining firmness, no sign of decay	Boonruang and others (2012)
Zeolite-based minerals	LDPE film	Kiwifruit	Extension of shelf-life up to 20 days at 4 °C by establishing equilibrium atmosphere, higher sensory quality	Ayhan (2016)
A natural hydroscopic mineral (not specified)	Commercial LDPE bags (by Peakfresh)	Broccoli	Less weight loss, maintenance of chlorophyll content and improvement of color and texture, shelf life of 12 days at 4 °C and 9 days at 10 °C	Jacobsson and others (2004)

Table 5: Antioxidant-releasing food packaging systems.

Active Substances	Matrix / Packaging Application	Incorporation to Matrix	Food Application	Benefit	Reference
BHT	LDPE films	Blow-extrusion	Fresh sierra fish (<i>Scomberomorus sierra</i>) fillets	Lower lipid oxidation and (peroxide value, TBARS, FFA) protein denaturation, less tissue damage, maintained firmness	Torres-Arreola and others (2007)
α -Tocopherol, BHT, BHA	Poly(lactide-co-glycolide) film	Solvent-casting	Dry whole milk and dry buttermilk powders	Improvement of oxidative stability (lower pentanal and heptanal content)	Van Aardt and others (2007)
α -Tocopherol	Multilayer film: HDPE/EVOH/LDPE	Twin-screw extrusion	Whole milk powder	Improvement of oxidative stability (lower pentanal, hexanal, and heptane content)	Granda-Restrepo and others (2009b)
	LDPE film	Solvent-casting	Corn oil	Improvement of oxidative stability (lower hexanal content)	Graciano-Verdugo and others (2010)
	Poly(lactic acid) film	Twin-screw extrusion	Soybean oil	Improvement of oxidative stability (lower peroxide value)	Manzanarez-López and others (2011)
Commercial mixtures of tocopherols	LDPE film	Melt-blending	MAP bluefin tuna fillets	Improvement of oxidative stability (lower TBARS)	Torrieri and others (2011)
	LDPE film	Twin-screw extrusion	Salmon (<i>Salmo salar</i>)	Improvement of oxidative stability (lower TBARS)	Barbosa-Pereira and others (2013)
Quercetin, ascorbic acid, ferulic acid, and green tea extract	EVOH film	Twin-screw extrusion	Brined sardines (<i>Sardina pilchardus</i>)	Improvement of oxidative stability (lower peroxide value and TBARS)	López-de-Dicastillo and others (2012b)
Catechin, quercetin	EVOH film	Solvent-casting	Fried peanuts, sunflower oil	Improvement of oxidative stability (lower hexanal content)	López-de-Dicastillo and others (2012a)
Citric acid	Cornstarch/linear LDPE film	Single-screw extrusion	Vacuum-packed ground beef (<i>Semimembranosus</i>)	Improvement of oxidative stability (lower TBARS) and color preservation (higher a* value)	Vargas Junior and others (2015)
Thymol, carvacrol, and eugenol	Corn-zein-laminated linear LDPE film	Solvent-casting	Vacuum-packed beef patties	Color improvement and inhibition of lipid oxidation (lower TBARS)	Park and others (2012)

Active Substances	Matrix / Packaging Application	Incorporation to Matrix	Food Application	Benefit	Reference
Resveratrol	LLDPE film	Direct addition to the polymer or pre-incorporation into montmorillonite clay, twin-screw extrusion	Beef	Oxidative stability improvement (lower TBARS)	Busolo and Lagaron (2015)
Green tea extract	Chitosan film	Solvent-casting	Pork sausages	Oxidative and color stability improvement (lower TBARS and higher L* value)	Siripatrawan and Noipha (2012)
Green tea extract, oolong tea extract, black tea extract	Protein film from distilled dried grains with solubles	Solvent-casting	Wrapped pork meat	Oxidative stability improvement (lower TBARS)	Yang and others (2016)
Oregano essential oil and green tea extract	Multilayer film: PET/PE/EVOH/PE	Film coating	MAP foal steaks (<i>longissimus dorsi</i>)	Color and oxidative stability improvement (lower metmyoglobin formation, TBARS)	Lorenzo and others (2014)
Green tea extract	Multilayer OPP film	Incorporation into lamination adhesive	Dark chocolate peanuts, milk chocolate cereals	Oxidative stability improvement (lower hexanal and higher pyrazine content)	Carrizo and others (2016)
Oregano extract	PP film	Film-coating	MAP beef steak	Oxidative stability improvement (lower TBARS) and color preservation (lower metmyoglobin content and higher a* value)	Camo and others (2011)
Oregano, rosemary extracts	PS tray	Film-coating	MAP lamb	Oxidative stability improvement (lower TBARS) and color preservation (lower metmyoglobin content and higher a* value)	Camo and others (2008)
Rosemary extract	PP film	Film-coating	MAP beef steak (<i>longissimus dorsi</i>)	Improvement of oxidative stability (lower TBARS) and color preservation (lower metmyoglobin content and higher a* value)	Nerin and others (2006)
	LDPE film	Film-coating	Vacuum-packed pork patties	Protection against HPP induced lipid oxidation (lower TBARS)	Bolumar and others (2016)
	LDPE film	Film-coating	Vacuum-packed pressurized chicken meat (breast and thigh) patties	Protection against HPP induced lipid oxidation Delay of lipid oxidation (lower TBARS)	Bolumar and others (2011)

Active Substances	Matrix / Packaging Application	Incorporation to Matrix	Food Application	Benefit	Reference
Natural extracts from brewery residual waste, rosemary extract	LDPE film	Film-coating	Beef	Oxidative stability improvement (lower TBARS)	Barbosa-Pereira and others (2014)
Barley husk extracts	LDPE film	Film-coating	Frozen blue shark (<i>Prionace glauca</i>)	Oxidative stability improvement (lower peroxide value, FFA, TBARS)	Pereira de Abreu and others (2011)
Encapsulated anthocyanins from wine grape pomace	Cassava starch film	Solvent-casting	Sunflower oil	Improvement of oxidative stability (lower peroxide value)	Stoll and others (2016)
Beet root residue powder	Gelatin capsule residue film	Solvent-casting	Sunflower oil	Improvement of oxidative stability (lower peroxide value)	Oliveira and others (2016)
Mango and acerola pulp	Cassava starch film	Solvent-casting	Palm oil	Improvement of oxidative stability (lower peroxide value, conjugated diene, and hexanal content)	Souza and others (2011)

(End of Table 5)

Table 6: Carbon dioxide emitters in food packaging systems based on the active substances sodium bicarbonate and citric acid.

Packaging Method with Emitter	Food Application	Quality Parameters	Benefit	Reference
MAP (70% CO ₂ , 30% N ₂) and vacuum	Cod	Microbial analysis, sensory analysis, drip loss, pH, trimethylamine (TMA) content	Extension of sensory shelf-life (from 7 to 11 days) and microbial shelf-life (vacuum + CO ₂ emitter: time for psychrophilic bacteria to reach log 6 CFU/g extended by 3 days), reduction in bacterial growth	Bjerkeng and others (1995)
MAP (60% CO ₂ , 40% O ₂)	Cod	Microbial analysis, drip loss, sensory analysis, pH	Maintenance of sensory and microbial shelf-life at lowered g/p ratio (g/p ratio 1.3/1.0 with CO ₂ emitter vs. 3.9/1.0 without CO ₂ emitter)	Hansen and others (2007)
	Salmon	Microbial analysis, drip loss, texture and sensory analysis, pH	Maintenance of sensory and microbial shelf-life at lowered g/p ratio (g/p ratio 1/1 with CO ₂ emitter vs. 3/1 without CO ₂ emitter)	Hansen and others (2009a)
MAP (60% CO ₂ , 40% N ₂) and vacuum	Cod	Microbial analysis, sensory analysis, drip loss	Improvement of initial freshness, extension of sensory and microbial shelf-life (Vacuum w/CO ₂ emitter: 2 days longer shelf-life, MAP w/CO ₂ emitter: 5 days longer shelf life).	Hansen and others (2016)
MAP (60% CO ₂ , 40% N ₂)	Reindeer meat	Microbial analysis, sensory analysis, pH, drip loss, antioxidant capacity, cooking loss	Reduction in drip loss (3.0 wt% in MAP packages without CO ₂ emitter, 1.0 wt% in MAP with CO ₂ emitter), reduction in bacterial growth (TVC at day 13-17: log 3-4 CFU/g with CO ₂ emitter, log 4-5 CFU/g without CO ₂ emitter)	Pettersen and others (2014)
MAP (100% CO ₂)	Chicken	Microbial analysis, pH, drip loss	Extension of sensory and microbial shelf-life; CO ₂ emitter facilitates packaging in 100% CO ₂ , reduction in drip loss (MAP 100 % CO ₂ : 7.5 wt% without CO ₂ emitter, 2.5 wt% with CO ₂ emitter)	Holck and others (2014)

Table 7: Antimicrobial food packaging systems - Essential oils

Active Substances	Matrix / Packaging Application	Microorganisms Tested	Food Application	Benefit	Reference
Cinnamon essential oil cinnamaldehyde	Multilayer active material, various polymers	<i>E. coli</i> O157:H7, <i>S. cerevisiae</i>	Tomato puree	Prevention of microbial growth: High activity against <i>E. coli</i> O157:H7 and <i>S. cerevisiae</i> , with reduction of both microorganisms in 3 log CFU/mL.	Gherardi and others (2016)
Cinnamon essential oil / β -cyclodextrin inclusion complex	Poly(lactic acid) nanofilm electrospinning	<i>E. coli</i> , <i>S. aureus</i>	Pork	Prolongation of shelf-life. MIC against both <i>E. coli</i> and <i>S. aureus</i> were approximate 1 mg/mL (corresponding CEO concentration 11.35 μ g/mL).	Wen and others (2016)
Cinnamaldehyde	Chitosan Reversible covalent Immobilization	<i>S. aureus</i> , <i>E. coli</i> and in milk inoculated with <i>L. monocytogenes</i>	Milk	Extension of microbiological shelf-life: Depending on the different time/temperature treatments, inhibition of the growth of <i>L. monocytogenes</i> up to 12 days under refrigeration conditions without causing any rejection among potential consumers due to the cinnamon smell.	Higueras and others (2015)
Carvacrol and thymol	Clay/PE polymer nanocomposite	Gray mold (<i>Botrytis cinerea</i>)	Strawberry	Prevention of <i>Botrytis</i> , synergistic antimicrobial effect IC ₅₀ was reduced from 40.4 mg/g (carvacrol only) to 13.2 mg/g (carvacrol:thymol 50:50). Effective inhibition of <i>B. cinerea</i> with one third of the total essential oils concentration without significant organoleptic alteration.	Campos-Requena and others (2015)
Oregano (<i>Lippia graveolens</i>) essential oil	Pectin edible coatings	Fungi	Tomatoes	Prevention of fungal decay and increase in antioxidant capacity. <i>In vivo</i> inhibition of the growth of <i>Alternaria alternata</i> from concentration of 25.9 g/L. Higher total phenols and antioxidant activity in coated tomatoes.	Rodriguez-Garcia and others (2016)
<i>Thymus moroderi</i> and <i>Thymus piperella</i> essential oil	Chitosan	Aerobic mesophilic bacteria, lactic acid bacteria, yeasts	Cooked cured ham, ready-to-eat meat	Prevention of aerobic mesophilic bacteria growth and lactic acid bacteria growth (2.6 and 2.1 log reduction after 7 days, respectively). Reduction of yeast population, could be 2 log CFU/g depending on the composition of the film	Ruiz-Navajas and others (2013); Quesada and others (2016)
Basil leaf essential oil	Fish protein isolate/fish skin gelatin-ZnO nanocomposite film	Psychrophilic bacteria, lactic acid bacteria, and spoilage	Sea bass slices	Shelf-life extension (12 days as compared to the control 6 days) demonstrated by sensory evaluation	Arfat and others (2015)

		microorganisms			
Extract of Allium spp.	PLA	Aerobic bacteria, Fungi	Ready-to-eat salads under controlled atmospheres	Extension of microbiological shelf-life. Reduction of Enterobacteriaceae up to 7.7 log units, 6.5% concentration of active agent effective up to 5 days of storage for aerobic bacteria, and even 7 days for molds.	Llana-Ruiz-Cabello and others (2015)
Vanillin	Starfish gelatin films	Bacteria <i>L. monocytogenes</i>	Crab stick	Decrease in <i>L. monocytogenes</i> growth on crabsticks with 0.05% in vanillin in film	Lee and others (2016)

Table 8: Antimicrobial food packaging systems - Enzymes and bacteriocins.

Active Substances	Matrix / Packaging Application	Microorganisms Tested	Food Application	Benefit	Reference
Enzymes					
Lysozyme	Whey protein isolate (WPI) film	<i>L. monocytogenes</i>	Smoked salmon	Inhibition of bacterial growth, extension of the shelf-life	Min and others (2005)
Lysozyme Lactoferrin	Lysozyme and lactoferrin incorporated into paper sheets	Total aerobic bacteria	Ready-to-eat thin-cut veal meat	Prevention of growth of microbiota	Barbiroli and others (2012)
Bacteriocins					
Nisin	Polyethylene (PE) film	<i>B. thermosphacta</i>	Beef carcass surface tissue	Inhibition of bacterial growth	Siragusa and others (1999)
	Coating on low-density polyethylene (LDPE) film	Microbiota, <i>M. luteus</i>	Milk: raw, pasteurized, UHT	Retardation of microbial growth	Mauriello and others (2005)
	Coating on LDPE film	<i>L. monocytogenes</i>	Cold-smoked salmon	Inhibition of bacterial growth	Neetoo and others (2008)
	Nisin incorporated into methylcellulose/hydroxypropyl methylcellulose coating on packaging films	<i>L. monocytogenes</i>	Hot dogs	Decrease of bacterial population	Franklin and others (2004)
	Nisin-coated paperboard	Total aerobic bacteria, Yeast	Pasteurized milk Orange juice	Improvement of microbial stability of milk and orange juice at 3 and 10 °C	Lee and others (2004)
Nisin/EDTA	Nisin or nisin/EDTA incorporated into PE or PE/polyethylene oxide films	<i>B. thermosphacta</i>	Beef	Reduction of bacteria on beef surface	Cutter and others (2001)
	Nisin/EDTA immobilized on the surface of the cellulose nanocrystal/chitosan nanocomposite films	Psychotropic bacteria, Mesophilic bacteria, <i>L. monocytogenes</i> , <i>E. coli</i>	Boneless pork loin meat	Inhibition of bacterial growth, extension of the shelf-life by more than 5 weeks	Khan and others (2016)
Nisin/citric acid/EDTA	Coating on polymeric films (polyvinyl chloride, nylon, linear LDPE)	<i>S. typhimurium</i>	Fresh broiler drumsticks	Inhibition of bacterial growth, extension of refrigerated	Natrajan and Sheldon (2000)

					shelf-life by 0.6 to 2.2 days	
Nisin/lacticin	Bacteriocin and polyamide coating on LDPE	Total aerobic bacteria, Coliform bacteria	Fresh oysters Ground beef	Retardation of microbial growth, extension of the shelf-life	Kim and others (2002)	
Nisin/enterocins /sakacin/potassium lactate	Active substances incorporated into interleaves	<i>Salmonella</i> spp.	Cooked ham	Reduction in bacteria counts	Jofré and others (2008)	
Nisin/enterocins /sakacin/potassium lactate	Active substances incorporated into interleaves	<i>L. monocytogenes</i>	Cooked ham	Inhibition of bacterial growth	Jofré and others (2007)	
Enterocins	Alginate, zein, and polyvinyl alcohol films	<i>L. monocytogenes</i>	Cooked ham	Delay and reduction of bacterial growth	Marcos and others (2007)	
Natamycin	Natamycin incorporated into cellulose polymeric films and laminated films	<i>P. roqueforti</i>	Gorgonzola cheese	Fungus inhibition, conservation of product	de Oliveira and others (2007)	
Lactocins	Lactocin-coated films: synthetic polymer multilayer films, and wheat gluten-based films	<i>L. plantarum</i> , <i>L. innocua</i>	Wieners	Inhibition of bacterial growth	Massani and others (2014)	
Pediocin	Pediocin incorporated into cellulose acetate film	<i>L. innocua</i> , <i>Salmonella</i> spp.	Sliced ham	Inhibition of bacterial growth	Santiago-Silva and others (2009)	

(End of Table 8)

Table 9: Antimicrobial food packaging systems – Antimicrobial Polymers.

Active Substances	Matrix / Packaging Application	Microorganisms Tested	Food Application	Benefit	Reference
ε-Polylysine	ε-Polylysine and/or sodium lactate incorporated in sorbitol-plasticized WPI films	Total bacteria, lactic acid bacteria	Fresh beef	Reduction of total flora, complete inhibition of lactic acid bacteria growth, extension of shelf-life	Zinoviadou and others (2010)
	Chitosan-incorporated LDPE film	<i>L. monocytogenes</i> , <i>E. coli</i> , <i>S. enteritidis</i>	Fresh beef (eye round roast)	Extension of red color shelf-life	Park and others (2010)
Chitosan	Chitosan, carboxymethyl cellulose and zinc oxide nanoparticles film	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>C. albicans</i>	Egyptian soft white cheese	Antibacterial and antifungal activity, extension of shelf-life	Youssef and others (2016)
	Chitosan-coated ethylene copolymer film	<i>E. coli</i> , <i>L. monocytogenes</i>	Beef and chicken meat exudates Turkey breast	Reduction in colony-forming units	Joerger and others (2009)
Chitosan/lauric arginate ester Chitosan/sodium lactate Chitosan/sorbic acid Chitosan/sodium lactate/sorbic acid Chitosan/lauric arginate ester/sodium lactate/sorbic acid	Coatings on PLA film	<i>L. innocua</i> , <i>L. monocytogenes</i> , <i>S. typhimurium</i>	Ready-to-eat meat (sliced turkey)	Reduction of <i>S. typhimurium</i> (undetectable level), reduction of <i>L. innocua</i> and <i>L. monocytogenes</i> , extension of shelf-life to 5 weeks	Guo and others (2014)
	Coatings on plastic film	<i>L. monocytogenes</i>	Ham steaks	Inhibition of bacterial growth on ham steaks for 12 weeks	Ye and others (2008a)
Chitosan/nisin Chitosan/sodium lactate Chitosan/sodium acetate Chitosan/potassium sorbate Chitosan/sodium benzoate	Cold-smoked salmon		Inhibition of bacterial growth on smoked salmon for at least 6 weeks	Ye and others (2008b)	
Chitosan/nisin, potassium sorbate/silver substituted zeolite	Antimicrobial agents incorporated into LDPE	Total aerobic mesophilic bacteria	Chicken drumsticks	Lower microbial counts and thiobarbituric acid reactive substance	Soysal and others (2015)

				(TBARS) values in the samples	
Chitosan/ethyl-N^α-dodecanoyl-L-arginate	Chitosan/glycerol films	<i>E. coli</i> , <i>S. aureus</i> , <i>L. monocytogenes</i> , <i>S. enterica</i> <i>P. putida</i> , <i>C. utilis</i> , <i>S. cerevisiae</i> var. <i>elipsoideus</i> , <i>C pinus</i> , <i>A. niger</i> , <i>P. chrysogenum</i> , <i>C. cladosporioides</i>	Chicken breast fillet	Inhibition of bacterial growth	Higueras and others (2013)

(End of Table 9)

Table 10: Antimicrobial food packaging systems - Organic acids, their derivatives and other organic compounds

Active Substances	Matrix / Packaging Application	Microorganisms Tested	Food Application	Benefit	Reference
Citric acid	Cornstarch/LLDPE film	Total bacteria	Minced beef	Reduction in bacterial growth compared with control samples (1 log CFU/g) after 10 days	Vargas Júnior and others (2015)
Sorbic acid and alga extract (<i>Fucus spiralis</i>)	PLA film	total aerobic bacteria, psychotrophic bacteria, Enterobacteriaceae	Megrim	Reduction in growth of psychotrophic bacteria (0.9 log CFU/g) compared with control samples, after 7 days. Better sensory properties (external odor, gill appearance, and flesh taste: acceptable after 11 days)	García-Soto and others (2015)
Sorbic acid	PVDC film	<i>L. monocytogenes</i> , total aerobic mesophilic and lactic acid bacteria	Beef bologna slices, cheddar cheese	Prevention of bacterial growth in beef bologna: <i>L. monocytogenes</i> up to 6.5 and 7.2 log lower with 1.5 and 3% sorbic acid films, respectively; and for LAB and mesophiles 4-6 log lower (28 days at 4 °C)	Limjaroen and others (2005)
Potassium sorbate	Thermoplastic starch/PBAT film	Total aerobic bacteria, total coliforms, <i>E. Coli</i> , <i>S. spp.</i> , <i>Salmonella spp.</i>	Restructured chicken steaks (low sodium content)	Reduction in initial <i>E. coli</i> counts (1.94 log CFU/g) to below detection limit throughout frozen storage (150 days).	Cestari and others (2015)
Potassium sorbate and/or sodium lactate	NaCl-brine before smoking and packaging in HDPE film	Total aerobic mesophilic bacteria, yeast and molds	Smoked rainbow trout fillets	Extension of shelf-life up to 4 weeks at 6 °C; Decrease in mesophiles (1.7-3 log CFU/g lower), yeast and mold growth	Kaya and others (2015)
Potassium metabisulfite (mixture pure and encapsulated)	PVC film	Total aerobic bacteria, <i>Salmonella spp.</i> , thermotolerant coliforms	Minimally processed cut-apples	Antimicrobial and antibrowning effect. Extension of shelf-life from 4 to 8 days at 8 °C; BI rated 50-60% compared to control (90%). TVC kept under 10 ⁶ CFU/g for 20 days at 4 °C and 12 days at 8 and 12 °C.	Foralosso and others (2014)
Oxidized regenerated cellulose	PCL film	<i>L. monocytogenes</i>	Sliced salami	Decrease in 50% for <i>Listeria monocytogenes</i> growth (from 8 log CFU/g) in 14 days at 4 °C	Sezer and others (2016)
Allyl isothiocyanate	Vapor phase in MAP	<i>P. aeruginosa</i>	Catfish fillets	Shelf life extension from 4 to 23 days in combination with MAP at 8 °C; inhibition of <i>P. aeruginosa</i> growth (<10 ⁴ CFU/g) during 23 days	Pang and others (2013)

Table 11: Antimicrobial food packaging systems – Nanoparticles

Active Substances	Matrix / Packaging Application	Microorganisms Tested	Food Application	Benefit	Reference
Ag/TiO ₂	LDPE/LLDPE	<i>A. flavus</i>	Cooked rice	10-fold reduction in <i>A. flavus</i> compared to the control (SEM images)	Li and others (2017)
Ag/TiO ₂	PE	Yeasts, molds, <i>B. subtilis</i> , <i>B. cereus</i>	Bread	Shelf-life extension up to 6 days	Mihaly Cozmuta and others (2015)
Ag	PVC	<i>E. coli</i> , <i>S. aureus</i> , <i>B. cereus</i> , <i>P. fluorescens</i>	Chicken breast fillets	Shelf-life extension for 2 days of storage, reduction in lipid oxidation	Azlin-Hasim and others (2016)
Ag/ZnO	LDPE	<i>E. coli</i> , <i>P. aeruginosa</i> and <i>L. monocytogenes</i>	Chicken breast fillets	Reduction in microbial growth (destruction of 99.99% of inoculated microorganisms.)	Panea and others (2014)
Ag and ZnO	LDPE	Yeasts and molds, total aerobic bacteria	Orange juice	ZnO: Shelf-life extension up to 28 days, Ag: Activity against yeasts and molds	Emamifar and others (2010)
ZnO	Active films (based on sodium alginate, calcium chloride and glycerol)	<i>S. typhimurium</i> , <i>S. aureus</i>	Poultry meat	Reduction in initial bacterial count (zone of inhibition from 16.6 to 29.4mm for <i>S. typhimurium</i> and 17-32.5mm for <i>S. aureus</i> - for NPs concentration from 1-4mg/m respectively)	Akbar and Anal (2014),
TiO ₂	LDPE	<i>Pseudomonas</i> spp., <i>R. mucilaginosa</i>	Fresh pears	Reduction in mesophilic bacteria and yeasts more than 2log CFU/g	Bodaghi and others (2013)
Cu	PLA	<i>Pseudomonas</i> spp. isolated from spoiled fiordilatte cheese	Fiordilatte cheese	Shelf-life extension up to 9 days	Conte and others (2013)