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## Plants: A natural solution to enhance raw milk cheese preservation?

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1 **Plants: a natural solution to enhance raw milk cheese**  
2 **preservation?**  
3

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19

20 **Abstract**

21

22 Plants have been traditionally used for centuries in cheese manufacturing, either for their aromatic  
23 properties or as technological auxiliaries (e.g. milk-clotting enzyme preparations, cheese wrappers).

24 Some of these plants are known to have antimicrobial and / or antioxidant properties and could also  
25 act as natural preservatives for raw milk and derived dairy products.

26 This review examined the traditional uses of plants in dairy processing, and then focuses on known  
27 antimicrobial and antioxidant properties of their extracts (e.g. maceration, decoction, essential oil).

28 Known effects of these plants on technological flora (starter cultures and microorganisms implicated  
29 in cheese ripening) were also summarized, and the potential for plant extracts used in combination  
30 with hurdle technologies was explored. Then, legal restriction and bioactivity variations from a culture  
31 media to a food matrix was reviewed: non-toxic bioactive molecules found in plants, extract  
32 preparation modes suitable with foodgrade processing restrictions, the role of the food matrix as a  
33 hindrance to the efficiency of bioactive compounds, and a review of food legislation. Finally, some  
34 commercial plant extracts for milk preservation were discussed.

35

36 **Keywords:** raw milk; cheese; plants; plant extracts; natural preservatives

37

38	<b>1. Introduction</b>
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52	<b>4. From traditional plants uses to innovative products for food conservation enhancement</b>
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54	4.2 Sample preparations and extractive techniques suitable for the food industry
55	4.3. Food legislation
56	4.4. Commercial innovative systems with plant extracts
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59	
60	

## 61 **1. Introduction**

62 The Mediterranean dairy sector is characterized by high production volumes and product  
63 diversification in all the Mediterranean countries. The Mediterranean countries comprise countries  
64 located in and around the Mediterranean Sea, that is to say Southern Europe (Gibraltar, Spain, France,  
65 Monaco, Italy, Malta, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Albania, Greece,  
66 Turkey), the Middle East (Syria, Cyprus, Lebanon, Israel, Palestine) and North Africa (Egypt, Libya,  
67 Tunisia, Algeria, Morocco). Mediterranean dairy products supply essential nutrients such as high  
68 biological value proteins, vitamins and minerals and are also attractive resources for the economic  
69 livelihood of marginal areas in hot climate countries (Pulina et al., 2018). However, in these countries,  
70 a warm climate and poor hygienic conditions, combined with inadequate refrigeration increase  
71 biohazard of raw milk. This poses health safety concerns but also economic and ecological problems.  
72 Indeed, during periods of milk overproduction (lactation period), prices can be lower and milk  
73 collection centers can become saturated, and so a part of the production is lost. In addition, the short  
74 shelf life of dairy product is an obstacle to distribution over long distances. Finally, in countries such  
75 as Egypt, a deeply rooted popular belief considers raw milk cheese as a safer product than heat-treated  
76 milk or pasteurized milk cheese. However, its consumption, especially by infants, can have dramatic  
77 consequences (Ayad, Omran, & El-Soda, 2006; Hammad, Hassan, & Shimamoto, 2015; Ayad et al.,  
78 2006).

79 In Europe, manufactured products made from raw milk are widespread and mainly located in the  
80 Mediterranean countries. In these countries, there are many traditional products e.g. cheeses,  
81 fermented milks, for which technological process prescribes the use of raw milk and very diverse  
82 technological procedures transmitted across centuries (Boyazoglu & Morand-Fehr, 2001). This  
83 diversification is officially recognized by the European Union through the attribution of the Protected  
84 Designation of Origin (PDO) label. Moreover, the number of consumers of raw milk is increasing in  
85 developed countries, as more people embrace the “organic” way of life and refuse to consume  
86 processed or preserved foods (Nero & Carvalho, 2018).

87 Recent studies have reviewed the microbiological hazard of raw milk and derived products (Claeys et  
88 al., 2013; Verraes et al., 2015). Milk contamination may be endogenous (e.g. *via* animal infection,

89 mastitis), or exogenous: the milk can be contaminated during milking *via* the udders and teats, but also  
90 by the environment and the material, i.e. the milking machine, bucket, or milk storage tank, and finally  
91 during the storage and transport. The major pathogenic bacteria found in raw milk cheese are *Listeria*  
92 *monocytogenes*, verocytotoxin-producing *Escherichia coli*, *Staphylococcus aureus*, *Salmonella sp.* and  
93 *Campylobacter sp.* (Boyazoglu & Morand-Fehr, 2001; Claeys et al., 2013; Hammad et al., 2015;  
94 Pesavento, Calonico, Ducci, Magnanini, & Lo Nostro, 2014; Verraes et al., 2015). As there is no heat  
95 treatment for raw milk cheese before production, these microorganisms are not killed during the  
96 process and can also cause foodborne disease outbreaks (EFSA & ECDPC, 2016; Lahti et al., 2017).  
97 However, small process modification during raw milk cheese production might have an effect on these  
98 microorganisms (growth inhibition, destruction, or even growth promotion). For example,  
99 fermentative bacteria (lactic acid bacteria) can inhibit or kill pathogenic bacteria in different ways,  
100 through nutritional and space competition or metabolite production (e.g. lactic acid, bacteriocins).  
101 Salting reduces the water activity, and inhibits some pathogenic bacteria growth. During ripening,  
102 changes in physico-chemical conditions, such as pH increase, might be more favorable for the growth  
103 of microorganisms including pathogenic ones. It is important to mention that the contamination on the  
104 surface of a cheese can also occur during ripening. On top of that, adding ingredients like plants or  
105 herbs could influence the evolution of the microorganisms in the cheese. Indeed, numerous countries  
106 produce cheeses with leaves and plants for their organoleptic properties, or for decorative or  
107 preservation purposes (Hayaloglu & Farkye, 2011).

108 The objective of this publication (Figure 1) is to review the traditional uses of plants for cheese  
109 making and their associated antioxidant and/or antimicrobial properties. A summarize of the scientific  
110 knowledge about the potential role of these plants for raw milk and cheese preservation is presented.  
111 This review also provide information on innovative herbal/legume systems (commercial or not) that  
112 have recently been developed for food/dairy products preservation or that could be used for such  
113 purposes. The last part reviews legislation for the use of these systems.

114

115

116

## 2. Traditional uses of plants for dairy processing

### 2.1. Addition of plants in milk, before cheese making

Plants have been traditionally used in many types of cheeses for flavoring. While the most commonly encountered ones are fresh cheeses mixed with aromatic herbs, all cheese families can be flavored with plants (Table 1). They are thus mixed directly with the cheese curd at the beginning of ripening, to add a particular flavour that will be enhanced during ripening process. Commonly used herbs and spices and other flavors added to cheeses include red, black and green peppers, thyme, cloves, cumin, parsley, paprika, onion/garlic and other plants such as shallots, chives; and less commonly tarragon, oregano, mustard seed or bran, nettles, nutmeg, basil, and horseradish. Traditionally, the amount rarely exceeds 1% of the curd (Hayaloglu & Farkye, 2011). Other flavors may include nuts, smoke, soot/ashes, cider, beer, wine and wine byproducts, mushrooms, olives and olive oil, sundried tomatoes, cocoa, bell pepper, or black pepper (Sicard, 2018)

While the addition of natural extracts to cheese curd for cheese fortification, e.g. with the intention of improving the level of antioxidant in the consumer's blood, is presently more and more investigated for health product development (Marchiani, Bertolino, Ghirardello, McSweeney, & Zeppa, 2015; Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2013), only a few studies concerning the role of extract additions in biopreservation processes are available. For example, the protective effects of a green tea extract against oxidation induced by light exposure were suggested (Huvaere et al., 2011).

Addition of black cumin oil to Domiati cheeses reduced the counts of the inoculated food borne pathogens during cold storage (Hassanien, Mahgoub, & El-Zahar, 2014; S. A. Mahgoub, Ramadan, & El-Zahar, 2013). Raw milk and its derivatives can also be preserved from pathogenic bacteria during cold storage by adding esterified legume proteins and 11S (glycinin) and 7S ( $\beta$ -conglycinin) soy globulin (S. Mahgoub, Osman, & Sitohy, 2011; A. Osman, Mahgoub, El-Masry, Al-Gaby, & Sitohy, 2014; A. O. Osman, Mahgoub, & Sitohy, 2013; M. Z. Sitohy, Mahgoub, & Osman, 2012). Moreover, extracts from plants used in these cheeses showed interesting bacteriostatic/bactericidal effects on target flora such as *Listeria monocytogenes in vitro*, or in other kinds of cheeses (see below).

144 However, these studies were often undertaken in culture media, thus neglecting the matrix effect that  
145 could hinder the active molecules release from plants, and without considering the effects on the  
146 positive endogenous raw milk flora. A better understanding of these aspects could be of particular  
147 interest, because, as seen in table 1, (i) most of the cheese that are concerned are soft curd cheeses,  
148 made with unpasteurized milk, with a short ripening time and considered as likely sources of  
149 microbiological hazards and (ii) as these plants have been traditionnaly used in cheese-making,  
150 sometimes for centuries, it seems that they are harmless to pro-technological microorganisms (figure  
151 1).

152

## 153 **2.2 Use of plant extracts for milk-clotting**

154 Since ancient times, plant extracts have been added to milk to facilitate the coagulation step in  
155 cheesemaking (Shah, Mir, & Paray, 2014). Cheeses made with vegetable coagulants instead of rennet  
156 are suitable for specific diets (vegetarianism) or religious restrictions (Halal, Kosher). However,  
157 nowadays, vegetable coagulant has often been replaced by animal rennet or microbial coagulant. In  
158 France, during the Middle ages, the coagulation of some variants of Jonchée Niortaise cheese was  
159 made using a thistle flower or artichoke extracts (Froc, 2007). The process has been preserved in  
160 several Mediterranean countries: today, Spain and Portugal are the most important producers of  
161 cheeses made with vegetable coagulant (Table 1).

162 *Cynara spp.* (in particular *C. cardunculus* var. *sylvestris*, also known as “wild cardoon”) is the most  
163 used vegetal coagulant (Shah et al., 2014). Usually, the flowers are harvested in the summer, before  
164 being air-dried. Despite an important variation of methods between cheesemakers, cardoon is added to  
165 milk using two major techniques : i) flowers are mixed to milk (at nearly 30°C), during a few minutes,  
166 and then milk is filtrated , ii) dry flowers are macerated in small amount of salted water and then the  
167 filtrat is added to the milk (Macedo, Xavier Malcata, & Oliveira, 1993).

168 With this method, milk-clotting is the result of the action of cardosins and cyprosins, a kind of aspartic  
169 proteases also known as phytepsins (EC 3.4.23.40), that have a high specificity towards  $\kappa$ -casein  
170 (Vairo Cavalli, Lufrano, Colombo, & Priolo, 2013). The use of serine and cysteine proteases from



171 plants for milk clotting has also been reported (Shah et al., 2014). Current uses in cheese making  
172 include caprifig extracts in Ficu and Cacioricotta (Faccia et al., 2012).

173

### 174 **2.3. Addition of plants on cheese, after coagulation**

175 While some cheeses are traditionally rolled into crushed herbs or spices such as garlic, pepper, sage,  
176 chives, rosemary, the whole plant is sometimes used directly on the cheese surface for flavouring  
177 and/or aesthetic purposes (Table 1). Among French cheeses, thyme or rosemary sprigs or even  
178 glasswort (Galette du Paludier) can be encountered. Wood, leaves have long been used in cheese  
179 processing and packaging around the globe. Aromatic effect most often appeared as a side-effect of  
180 that packaging process while the initial intention was cheese preservation (Froc, 2007). Chestnut,  
181 gentian, avizzo, or vine leaves, for example, were (or are still) used as cheese interleaves (Cantin,  
182 Gaudin, & Leser, 2013), as moisture absorbers or for cheese wrapping. In France, Banon goat cheese  
183 is ripened wrapped in chestnut leaves (Décret du 23 juillet 2003 relatif à l'appellation d'origine  
184 contrôlée « Banon », 2003) which brings specific flavours thanks to anaerobic fermentation and to the  
185 tannin-rich rot-proof leaves. These traditionnal uses participate in the taste of the final product  
186 (Collectif, 1998; Froc, 2007; Sicard, 2018), which directly suggests molecule migrations from the  
187 organic matter to the cheese surface and/or curd. In the U.K., the Cornish Yarg is wrapped in nettle or  
188 wild garlic leaves to form an mouldy edible rind. In Italy, Pecorino is sometimes covered with a fresh  
189 walnut leaf, Ubriaco with a fig leaf and Seirass with hay for decorative purposes (Sicard, 2018).  
190 Little is known concerning possible migration of molecules, such as phenolic compounds from the leaf  
191 to the cheese, and concerning their potent action as natural preservatives during cheese storage and  
192 ripening. Terpenes arising from the surface of mint leave springling, during the manufacture of the  
193 cheese, were detected in Halloumi cheese curd (Papademas & Robinson, 2001). Their role as  
194 flavouring molecules is reported, but while the role of mint as a preservative in this cheese is  
195 traditionally claimed, as the cheese is entirely covered in folded mint leaves during the ripening period  
196 (Sicard, 2018), it has never been scientifically investigated.

197

### 198 **3. Biopreservation of milk and cheeses with plants**

199 Numerous plants traditionally added in dairy products have been studied for their anti-oxidant and  
200 anti-microbial effects. This part summarizes findings on the plants whose efficiency as been  
201 demonstrated (Table 2).

202

#### 203 **3.1. Dairy products with addition of essential oils**

204 As the demand for natural preservatives increases, essential oil addition in cheese was particularly  
205 explored (Table 2). For example, *E. coli* O157:H7 and *L. monocytogenes* growth were inhibited in feta  
206 cheese by adding essential oil of thyme (at a dose of 0.1 mL/100 g) (Govaris, Botsoglou, Sergelidis, &  
207 Chatzopoulou, 2011) or oregano (0.1 or 0.2 mL/100 g) (Govaris et al., 2011). Antimicrobial activity of  
208 these 2 essential oil was also demonstrated in fresh goat cheese (Zantar et al., 2014). Original  
209 approaches, such as the use of antimicrobial sachets containing rosemary and thyme oils, designed to  
210 enhance the shelf-life of shredded mozzarella, were also reported (Han, Patel, Kim, & Min, 2014).

211 The recent review of Khorshidian et al. (Khorshidian, Yousefi, Khanniri, & Mortazavian, 2018) on  
212 preservation of cheese with essential oils can be consulted for further information.

213

#### 214 **3.2. Dairy products with addition of plant extract other than essential oil**

215 In spite of the proven antibacterial effect of essential oil for cheese preservation, several factors limit  
216 their application: intense flavour and potential interactions with food components may be an hindrance  
217 to their efficiency. Other concerns include their price (Calo, Crandall, O'Bryan, & Ricke, 2015;  
218 Hyldgaard, Mygind, & Meyer, 2012; Perricone, Arace, Corbo, Sinigaglia, & Bevilacqua, 2015), and  
219 their potential toxicity at high doses despite their GRAS status. For these reasons, recent studies on  
220 dairy product preservation have focused on the effects of plant extracts other than essential oil (Table  
221 2). Rosemary extract was thus successfully used as an antioxidant for the lipid preservation of cow  
222 milk enriched with fish oil (Qiu, Jacobsen, & Sørensen, 2018). Rosemary extract also inhibited the  
223 growth of *L.monocytogenes* ( $4 \times 10^5$  CFU.mL<sup>-1</sup>) incubated at 37°C for 24 hours in cheddar base media  
224 at a concentration of 750 µg.mL<sup>-1</sup> (Tayel, Hussein, Sorour, & El-Tras, 2015). Rosemary alcoholic

225 extracts enhanced the oxidative stability of butter at temperatures of 60 and 110°C, when added at  
226 concentrations such as 400 mg of phenolic compounds per kg of butter (Santos, Shetty, & da Silva  
227 Miglioranza, 2014). A clove extract (625 µg.mL<sup>-1</sup>) inhibited a population of *L.monocytogenes* (4x10<sup>5</sup>  
228 CFU.mL<sup>-1</sup>) incubated at 37°C for 24 hours in cheddar based media (Tayel et al., 2015). In the same  
229 study, an oregano extract (950 µg.mL<sup>-1</sup>) yielded similar results.

230 Other extracts of plants used in cheesemaking have shown antimicrobial properties against foodborne  
231 pathogens, like *Thymus vulgaris* (Thymus) (Kozłowska, Laudy, Przybył, Ziarno, & Majewska, 2015;  
232 Mostafa et al., 2018), *Olea europea* (oliv tree) (Ahmed, Rabii, Garbaj, & Abolghait, 2014; Aouidi,  
233 2012; Botsoglou, Govaris, Ambrosiadis, Fletouris, & Papageorgiou, 2014; Sudjana et al., 2009) or  
234 *Urtica doica* (nettle) (Aksu & Kaya, 2004; Alp & Aksu, 2010; Alp Erbay, Dağtekin, Türe, Yeşilsu, &  
235 Torres-Giner, 2017), but to our knowledge, these extracts have not been tested in dairy products. On  
236 the other hand, an extract from a plant belonging to the genius *Thymus*, *Thymus mastichina*, was able  
237 to inhibit the development of spoilage microorganism on the cheese surface (F. Carvalho et al., 2018).  
238 Also, an extract of oil mill wastewater from olives, rich in phenolic coumpounds, was tested to  
239 enhance the preservation of “Fior di latte” cheese. The phenols retarded the growth of the spoilage  
240 bacteria *Pseudomonas fluorescens* and *Enterobacteriaceae*, resulting in a cheese longer shelf life  
241 (Roila et al., 2019).

242 The use of plant extracts, as preservatives can also be a source of innovative products by creating new  
243 tastes as a side effect. Low fat Kalari (Indian hard and dry cheese) was sprayed with 0%, 2.5% and  
244 5.0% pine needles extract (*Cedrus deodara*) and aerobically packaged in low-density polyethylene  
245 pouches. This resulted in lower thiobarbituric acid reactive substances, free fatty acid (% oleic acid)  
246 values, and lower total plate, counts of psychrophilic bacteria, yeast and mould (Mahajan, Bhat, &  
247 Kumar, 2016). Addition of *Inula britannica* flower extract during cheddar-type cheese making  
248 increased antioxidant properties (Lee, Jeewanthi, Park, & Paik, 2016). Clove (*Syzygium aromaticum*)  
249 extract increased the stability of cheese against lipid oxidation (Shan, Cai, Brooks, & Corke, 2011)  
250 and was also efficient against *L. monocytogenes*, *S. aureus*, and *Salmonella enterica* in cheese at room  
251 temperature (Shan et al., 2011). Finally, fresh ovine Greek cheese was supplemented with saffron,

252 resulting in a significant decrease of the total aerobic bacteria counts, with an antibacterial activity  
253 against coliform and enterococci groups (Aktypis et al., 2018).

254 Another possibility is to use plant proteins as alternative food preservatives (Nielsen, 1985). Active  
255 peptides were produced from plant seeds such as peas (Aluko, 2008) soy bean (Chen, Yang, Suetsuna,  
256 & Chao, 2004; Wu & Ding, 2002), rice, sunflower and wheat (Guang & Phillips, 2009). These active  
257 peptides were found to be good sources of antimicrobial, antioxidants and functional agents.

258 Several classes of plant proteins and their fractions with antibacterial and/or antifungal properties have  
259 been isolated, characterized and applied as antimicrobial agents in food systems (A. Osman et al.,  
260 2014, Ali Osman, Abbas, Mahgoub, & Sitohy, 2016; M. Sitohy, Doheim, & Badr, 2007, Sitohy,  
261 Mahgoub, Osman, El-Masry, & Al-Gaby, 2014; Osman, El-Araby, & Taha, 2016). The minimum  
262 inhibitory concentrations (MICs) of 7S and 11S globulins isolated from cowpea seeds ranged between  
263 10 to 200  $\mu\text{g}\cdot\text{mL}^{-1}$  against *L. monocytogenes*, *Listeria ivanovii*, *S. aureus*, *Streptococcus pyogenes*,  
264 *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *E. coli* and *Salmonella sp.* Supplementing 11S  
265 globulin at both concentrations 50 and 100  $\mu\text{g}/\text{g}$  to minced meat showed considerable decreases in  
266 viable bacterial count, psychrotrophs and coliforms (Abdel-Shafi, Al-Mohammadi, Osman, Enan,  
267 Abdel-Hameid, & Sitohy, 2019). Therefore, further studies are needed to use antimicrobial natural  
268 peptides as food preservatives.

269

### 270 **3.3. Dairy products with raw plant additions**

271 Studies concerning the potential of whole leaves and dried herbs for cheese preservation are scarce.  
272 The commercial potential of cheese covered with lard and dehydrated rosemary leaves was explored  
273 (Marinho, Bersot, Nogueira, Colman, & Schnitzler, 2015) for their antioxidant effects. To date, the  
274 only study exploring health-related effects of a leaf-wrapped cheese concerned the Banon cheese. The  
275 Banon cheese curd was investigated for antiproliferative activity against the HL-60 human  
276 promyelocytic leukemia cell line, but no significant effect was observed, while other investigated goat  
277 cheeses revealed more interesting profiles (Yasuda et al., 2012).

278 Carochó et al. (Carochó et al., 2016) compared the effect of the incorporation of decoctions and  
279 dehydrated basil in Serra da Estrela cheese. Basil leaves provided antioxidant activity to the cheeses,

280 reduced the moisture and preserved the unsaturated fatty acids and proteins; while the basil decoction  
281 showed an antimicrobial effect against eight bacterial and eight fungi strains in culture media. These  
282 bioactivities were not studied in cheese, though. Josipovick (Josipović et al., 2015) added fresh or  
283 dried parsley, dill, pepper, garlic and rosemary to cottage cheese. This plant addition did not reduce  
284 the concentration of foodborne pathogens, contrary to the plant extract. Soft cheese supplemented with  
285 either 1% garlic or 1% clove powders showed no improvement in antimicrobial effects against  
286 *L. monocytogenes* after one or two weeks at 37°C and at 4°C (Leuschner & Ielsch, 2003).

287 The effect of *Matricaria recutita* L. (chamomile) on cheese preservation was tested by adding a  
288 chamomile powder or a decoction (Caleja et al., 2015). Both additions similarly improved the  
289 antioxidant activity of plain cottage cheese without modifying the nutritional characteristics or fatty  
290 acids profiles. After 14 days of storage, only the control cheese showed visual microbial degradation.  
291 The bioactivity of the chamomile decoction was tested in cultured media showing antimicrobial  
292 activities against 16 bacterial and fungi strains (which can be attributed to the presence of phenolic  
293 compounds).

294 Another strategy is to directly incorporate plants to the cattle's diet. A rosemary leaves distillate was  
295 introduced in the diet of the Murciano-Granadina goats and the resulting effects on the phenolic  
296 profile of the milk was investigated. While neither yield nor quality were impaired, flavonoids and  
297 phenol diterpenes could be detected in relevant quantities in the goat milk (Jordán, Moñino, Martínez,  
298 Lafuente, & Sotomayor, 2010). However, the same kind of experiment performed with thyme leaves  
299 distillate resulted in a slightly impaired clotting ability, and the addition of thyme leaves did not show  
300 any inhibition of the mesophilic aerobic flora and enterobacteria growth between the control and the  
301 cheeses made from such milk (Boutoial et al., 2013).

302

### 303 **3.4. Biological activities of plant extract used for milk-clotting**

304 As previously described, some plant extracts, such as *Cynara cardunculus* var *sylvestris* flower  
305 extracts, may be used for milk clotting. (Ben Amira et al., 2017). Besides this application, some  
306 studies have shown the antioxidant and antimicrobial effect of different organs of the *C. cardunculus*

307 species, due to phenolic compounds with bioactive properties. The three main *C. cardunculus* taxa are  
308 the domestic cardoon (*C. cardunculus* var. *altilis* DC), the globe artichoke (*C. cardunculus* var.  
309 *scolymus* L.) and the wild cardoon (*C. cardunculus* var. *Sylvestris* (Lamk) Fiori).  
310 Falleh et al. (Falleh et al., 2008) compared extracts from domestic cardoon leaves, flowers and seeds.  
311 The phenolics/flavonoids contents of leaves and seeds were similar (approximately 14 mg GAE g<sup>-1</sup>  
312 DW) and 2 times higher than those in flowers. Seed extracts displayed the highest DPPH<sup>•</sup> followed by  
313 leaves, and flowers; IC<sub>50</sub> values were 23, 53 and 118 µg.mL<sup>-1</sup> respectively. In contrast, leave extract  
314 showed the highest capacity to quench superoxide compared to seeds and flowers, IC<sub>50</sub> values were 1.  
315 6 and 9 µg.mL<sup>-1</sup> respectively. Only an extract from leaves was tested for antimicrobial activity,  
316 showing growth inhibition of *S. aureus* and *E. coli*. Kukić et al. (Kukić et al., 2008) also showed the  
317 antioxidant and antimicrobial effects of cardoon leave extracts. Besides their strong antioxidant  
318 activity, the cardoon inflorescences showed antibacterial activity against Gram-positive strains, the  
319 lowest MIC being observed for *L. monocytogenes* (Dias et al., 2018). However, this antimicrobial  
320 effect was not found in food media when comparing Ewes' cheese produced with plant coagulant from  
321 cardoon *C. cardunculus* (21 g.100 L<sup>-1</sup>) and cheese produced with a calf rennet (Galán, Cabezas, &  
322 Fernández-Salguero, 2012). Throughout the ripening of the cheeses, no significant differences in  
323 microbial counts were observed between the ones made with these two types of coagulant. It can also  
324 be noted that Silva et al. (Silva, Pihlanto, & Malcata, 2006) studied ovine and caprine cheeselike  
325 systems manufactured with proteases from *C. cardunculus* and showed that it is a source of peptides  
326 with antioxidant activities.

327

### 328 **3.5. Effect of plant extract on pro-technological flora**

329 One drawback of using plant extracts as preservatives is that cheese is a fermented product containing  
330 a living flora, and thus such preservatives have an action on the pathogenic flora while sparing  
331 microorganisms implicated in milk coagulation and ripening.

332 *Thymus vulgaris* essential oil affected *S. aureus*, *L. monocytogenes* growth (1.3 log CFU.mL<sup>-1</sup>  
333 reduction at 10°C for 24h), but also the mesophilic starter co-culture in cheese-mimicking models

334 (cheese-based broth and in a semi-solid (*coalho*) cheese) (R. J. de Carvalho et al., 2015). Some  
335 essential oils met this criterion successfully, though : a rosemary essential oil (215mg.L<sup>-1</sup> in sheep milk  
336 cheese) inhibited *Clostridium ssp.* during the 5 month ripening period while not reducing the  
337 population of lactic acid bacteria (Moro, Librán, Berruga, Carmona, & Zalacain, 2015). *Cuminum*  
338 *cuminum* essential oil (0, 7.5, 15 and 30 µL.100mL<sup>-1</sup> in cow milk) used in synergy with *Lactobacillus*  
339 *acidophilus* strains also inhibited *S. aureus* in Iranian white brined cheese (Sadeghi, Akhondzadeh  
340 Basti, Noori, Khanjari, & Partovi, 2013). Oregano essential oil (200 mg.L<sup>-1</sup> in cow milk) impeded the  
341 growth of enterobacteria in a traditionnal Argentinean cheese while not affecting starters (Marcial et  
342 al., 2016). Thyme or sage essential oils added at doses of 1500 mg.kg<sup>-1</sup> to Fior di Latte cheese  
343 inhibited *Pseudomonas spp.* and coliforms stored at 10°C for 6 days while not reducing the population  
344 of lactic acid bacteria (Gammariello, Di Giulio, Conte, & Del Nobile, 2008). Grape seed extract of  
345 *Vitis vinifera* showed important anti- *L. monocytogenes* effects (Rhodes, Mitchell, Wilson, & Melton,  
346 2006), while the addition of grape seed extract of *Vitis vinifera* did not affect yoghurts *Lactobacilli*  
347 count (Chouchouli et al., 2013). Galindo et al. (Galindo-Cuspinera, Westhoff, & Rankin, 2003) studied  
348 the antimicrobial properties of a commercial Annato extract against pathogenic, lactic acid and  
349 spoilage microorganisms. Indeed, Annato is an orange-red condiment and food coloring extracted  
350 from the achiote tree (*Bixa orellana*), and added to obtain the color of numerous cheeses e.g. Cheddar,  
351 Mimolette, Edam and Livarot. The effect was very variable between studied strains. An inhibitory  
352 effect on pathogenic *Bacillus cereus*, *Clostridium perfringens*, and *S. aureus*, with MICs values of  
353 0.08, 0.31, and 0.16% (vol/vol) was respectively observed. Higher concentrations of Annato (0.63%,  
354 vol/vol) were needed to inhibit the growth of pro-technological *Streptococcus thermophilus*,  
355 *Lactobacillus casei* subsp. *casei* and *Lactococcus lactis*. However, at this concentration, the growth of  
356 *L. monocytogenes* and *Enterococcus durans* were not inhibited (MICs: 1.25 and 2.5%, vol/vol,  
357 respectively). No activity was detected against *Lactobacillus plantarum*, *Bifidobacterium bifidum*,  
358 yeasts, or selected Gram-negative bacteria.

359

### 360 **3.6. Synergic effect and hurdle technology**

361 To enhance the effects of plant extracts, combinations with other preservation technologies have been  
362 studied. For example, supercritical fluid thyme extracts containing carvacrol (190.44–609.57  $\mu\text{g}\cdot\text{mL}^{-1}$ )  
363 reduced  $\text{CFU}\cdot\text{g}^{-1}$  *L. monocytogenes* by 1.68 log in cheeses when combined with a high pressure  
364 treatment (Bleoancă et al., 2016). The addition of 3% of cayenne or of 9% of dry green pepper extract  
365 (obtained from a maceration in 70% ethanol) to Kareish cheese during manufacture reduced the *S.*  
366 *aureus* population from  $10^8$   $\text{CFU}\cdot\text{g}^{-1}$  to undetectable levels within two days of storage at  $4^\circ\text{C}$  (Wahba,  
367 Ahmed, & Ebraheim, 2010).

368

## 369 **4. From traditional plants uses to innovative products for food conservation** 370 **enhancement**

371 Tradition provides interesting semi-empiric knowledge, and is particularly helpful in the choice of  
372 plants to use for bio-guided essays. Nevertheless, scientific investigations are required to achieve  
373 innovative preservation solutions. Compound identification and characterization, extraction process  
374 optimization and tests for undesirable effects are needed. The specificities of the food industry in  
375 terms of stability and toxicity, for example, must be investigated. Also, these solutions must comply  
376 with current food legislation or provide sufficient evidence to modify it.

377 In this part, an overview of plant compound families with a potential as food preservative, foodgrade  
378 extraction techniques and the food legislation constraints, is presented.

379

### 380 **4.1 Plant bioactive compounds families with potential use as food** 381 **preservatives**

382 Most of the compounds from plants that are of interest in biopreservation solutions are secondary  
383 metabolites with various functions in plant survival, among which is the antimicrobial activity. Plant  
384 metabolite content varies according to the taxa, the organ, the stage of growth and development, the



385 season, and the stress conditions, for example. Metabolites are classified into different categories  
386 depending on the chemical structure and the main ones are presented in Table 3.

387 The most abundant literature concerns phenolic compounds. They have important roles fighting plant  
388 pathogens and ultraviolet radiations, and so can be good candidates for antimicrobial and antioxidant  
389 activities. They are compounds with one or more hydroxyl groups attached to a phenyl moiety. Based  
390 on their chemical structure, they can be divided into flavonoids and non-flavonoïds phenolics. Other  
391 secondary metabolites with reported antibacterial and/or antioxydant properties consist of waxes, fatty  
392 acids, alkaloids, terpenoids, glycosides and phytosterols (Da Silva, Rocha-Santos, & Duarte, 2016,  
393 Mogoşanu, Grumezescu, Bejenaru, & Bejenaru, 2017; Hugo & Hugo, 2015). It is generally accepted  
394 that such compounds are more efficient against Gram-positive than Gram-negative bacteria (Pisoschi  
395 et al., 2018; Hintz, Matthews, & Di, 2015).

396 Antimicrobial peptide also represents a promising tool for enhancing the shelf-life of food (Johnson et  
397 al., 2017). Generally, their mode of action towards pathogens consists in electrostatic interaction,  
398 membrane permeabilizing and then disruption (Rai, Pandit, Gaikwad, & Kövics, 2016). Important  
399 structural diversification of antimicrobial peptides from plants and their associated antibacterial  
400 activity provide lots of potential natural compounds for food preservation (Salas, Badillo-Corona,  
401 Ramírez-Sotelo, & Oliver-Salvador, 2015).

402 However, an extract showing an antimicrobial effect in a culture medium will not necessarily have an  
403 effect in a food media, because of the structure's complexity (Calo et al., 2015; Hyldgaard et al., 2012;  
404 Perricone et al., 2015). For example, antimicrobial effect could be lowered due to solubilization of  
405 hydrophobic active molecules in lipidic phases, whereas microorganisms are present in aqueous  
406 phases. Proteins may also interact with phenolic compounds (Calo et al., 2015; Hyldgaard et al., 2012;  
407 Perricone et al., 2015). Finally, during the different steps of cheese production,  $a_w$ , pH, microflora  
408 composition, temperature and nutrient composition will change, having an influence on the biological  
409 activity of plant extracts in food. Therefore, a fine understanding of the chemical nature of plant  
410 extracts, of food matrix characteristics and possible interactions with dairy food components of  
411 extracted molecules is a prerequisite.

412

## 413 **4.2 Sample preparations and extractive techniques suitable for the food** 414 **industry**

415 Different techniques exist to extract compounds from plants and several parameters, such as  
416 temperature, pressure and solvent composition might be set. In this part, common extractive  
417 techniques are discussed with a differentiation between extraction for essential oil and other extraction  
418 (mainly solid-liquid extraction).

419 Prior to extraction, several sample preparations are used. Generally, dried samples are preferred to  
420 fresh samples because of metabolites deterioration during storage. However, cases were reported for  
421 *Moringa oliefera* leaves for which no significant differences with total phenolic contents were  
422 observed between dry and fresh samples whereas dried samples contain more flavonoids (Vongsak et  
423 al., 2013).

424 The contact surface between the organic material and the solvent highly impacts the extraction yield  
425 and so, a powdered sample is preferred to a grinded one. This preparation leads to small and  
426 homogenized organic particles. It was shown that particle sizes smaller than 0.5 mm are ideal for an  
427 efficient extraction (Thermo scientific, 2013). The most common method is maceration, for which no  
428 apparatus is needed. This method uses small amounts of solvent and is inexpensive. The main  
429 disadvantage is a moderate yield.

430 Soxhlet extraction is a continuous extractive technique for which no successive extractions are needed  
431 to fully drain the secondary metabolites from the organic material, it is also very simple and cheap  
432 (Luque de Castro & García-Ayuso, 1998). Disadvantages of this technique are a long extraction time  
433 and a high risk of thermal decomposition since solvent temperatures are up to their boiling point  
434 during the extraction (Wang & Weller, 2006).

435 Introduced in the 90s, pressurized liquid extraction is characterized by high temperature (from 50 to  
436 200 °C) and high pressure (from 500 to 3000 psi), mainly to keep the solvent liquid, resulting in high  
437 performance (yield, quantities of solvent, duration) (Azmir et al., 2013). A high temperature disturbs  
438 weak interactions (Van der Walls, hydrogen bond and dipolar interaction) and decreases solvent  
439 viscosity. High pressure increases contact between solvent and matrices. Some limitations for the

440 technique include its cost and the degradation of thermo sensible compounds (Mustafa & Turner,  
441 2011).

442 Enzyme-assisted extraction is a recently developed technique that might be considered sustainable  
443 since it uses mainly water. Applied temperatures are generally below 90°C, and less solvent is needed.  
444 It consists in the use of selected enzymes (e.g. cellulase, pectinase, hemicellulose) to disrupt cellular  
445 integrity and/or hydrolysis metabolites bound to the membrane. The efficiency of this technique  
446 depends strongly on the cell-wall architecture, and so it seems attractive for sea-weed whose cell walls  
447 and cuticles are chemically and structurally more complex and heterogeneous than those of land plants  
448 (Wijesinghe & Jeon, 2012). This technique has successful applications on commercial compounds  
449 such as the extraction of carotenoids from marigold flowers (Barzana et al., 2002), the simultaneous  
450 extraction of glucovanillin from green vanilla pods and the transformation into vanillin (Ruiz-Terán,  
451 Perez-Amador, & López-Munguia, 2001) and lycopene extraction from tomatoes peel (Dehghan-  
452 Shoar, Hardacre, Meerdink, & Brennan, 2011). Numerous commercial compounds extracted using  
453 enzyme-assisted extraction are listed in recent reviews (Mustafa & Turner, 2011; Puri, Sharma, &  
454 Barrow, 2012; Wijesinghe & Jeon, 2012).

455 Essential oils are composed of volatile and lipophilic compounds, which are mainly lightly  
456 hydroxylated terpenoid and low molecular weight aromatic compounds. They can be obtained by  
457 different means, among which hydrodistillation, steam distillation, maceration in apolar solvent and  
458 supercritical extraction (Tyśkiewicz, Gieysztor, Konkol, Szałas, & Rój, 2018). Hydrosols, which are  
459 aqueous products of hydrodistillation (also known as floral waters), might be considered too, since  
460 they usually are food grade products, and some (cumin, clove, garlic, mustard, oregano, rosemary,  
461 sage and thyme hydrosols) exhibited antimicrobial activities against food relevant strains (D'Amato,  
462 Serio, López, & Paparella, 2018). Hydrodistillation using a Clevenger apparatus is a common method  
463 for essential oil extraction because it is cheap and easy to use, but some of its disadvantages are a low  
464 yield, losses of volatile compounds, and long extraction times. Moreover, the degradation of  
465 unsaturated compounds, partial hydrolysis and deterioration due to heat-sensitive compounds might  
466 occur (Puri et al., 2012). Nevertheless, recent innovations improving yield and decreasing the

467 extraction time have been reported. These were hydrodistillation coupled with ultrasonic extraction  
468 and ohmic hydrodistillation. During ohmic hydrodistillation, an alternative current is passed through  
469 the round bottom flask to generate heat within the medium (Gavahian, Farahnaky, Shavezipur, &  
470 Sastry, 2016).

471 Most expensive, the supercritical fluids extraction (SFE) can be performed at temperatures around 313  
472 K thereby preserving original oil composition and properties. SFE is mostly performed with carbon  
473 dioxide (CO<sub>2</sub>) because it has low critical pressure (73.8 bar) and temperature (31.1°C). In addition, it  
474 is relatively non-toxic, nonflammable, and relatively cheap (Sodeifian, Sajadian, & Saadati Ardestani,  
475 2016, Ivanovic, Mistic, Zizovic, & Ristic, 2012), nevertheless other solvents (or co-solvent), including  
476 water, may be used (Da Silva et al., 2016). SFE is valuable for the food industry especially for  
477 products such as de-caffeinated or de-alcoholized beverages, zero-fat potato chips, and others  
478 (Brunner, 2005).

479 The application of ultrasound during the extraction (UAE) process is widely used to increase yield and  
480 to decrease duration. Under some conditions, the ultrasonic capillary effect increases the depth and the  
481 velocity of the penetration of the extraction solvents into canals and pores. Moreover, during the UAE,  
482 disruptions of biological membranes may occur, therefore facilitating the release of extractable  
483 compounds. Comparing the extraction rate of chlorophylls between the UAE and maceration process,  
484 a linear increase is obtained at the beginning of the UAE, corresponding to a direct solubilization of  
485 chlorophylls (ref "a ajouter partie ultrasons"). Fragmentation of friable solids resulting from ultrasonic  
486 cavitation has been identified by several authors. In addition, particle size may decrease during the  
487 application of ultrasound therefore increasing the surface exchange. Fragmentation can be due to inter-  
488 particle collisions, and from shockwaves created from collapsing cavitation bubbles in the liquid. The  
489 ultrasonic capillary effect (UCE) refers to the increase in the depth and velocity of the penetration of  
490 the liquid into canals and pores under some of the conditions of ultrasonication (Dai & Mumper, 2010,  
491 Vilku, Mawson, Simons, & Bates, 2008). Ultrasound-assisted extraction is cheap and might be  
492 applied to a small and large scale of plants.

493

### 494 **4.3. Food legislation**

495 Raw milk can be contaminated by various important pathogenic bacteria leading to safety risks for  
496 consumers. High level of hygienic conditions during milking are required to minimize microbial risk.  
497 Unfortunately, such conditions are not sufficient to guarantee safety for consumers (Willis et al.,  
498 2018). Not only taste, but also tradition, cultural norms, socio-economic status, health perception and  
499 risks contribute to the acceptance of raw milk products by consumers. Preferences for raw- *versus*  
500 pasteurized-milk products vary between countries, with trends emerging, such as the preference for  
501 raw-milk products in Southern Europe and for pasteurized-milk products in Northern Europe (Nero &  
502 Carvalho, 2018). These different consumption cultures impact legislations all around the world. For  
503 example, it is illegal to sell raw milk as food in Australia, Canada and in some States of the United  
504 States of America (USA) (Baars, 2019), while raw milk sale is authorized in the European Union,  
505 although some differences in law interpretation between country members should be noted. In some  
506 areas, like England or some American States , raw milk can be sold but only directly from the  
507 producer to the consumer (Baars, 2019, Willis et al., 2018). In Mediterrean countries like Tunisia or  
508 Egypt, raw milk consumption is a tradition and food regulations are generally harmonized with EU  
509 food regulation (CE) N°178/2002. Although some countries allow raw milk sales, to date, heat  
510 treatment (pasteurization or sterilization) is highly recommended by governments, regulatory  
511 authorities and/or public health practitioners in order to reduce the risk of foodborne illness  
512 (Muehlhoff & FAO, 2013, Codex Alimentarius Commission, 2011).

513 Similarly to raw milk, raw-milk derived products are considered as “risky foods.” Heat treatment  
514 applied to milk (pasteurization or sterilization) leads to an alteration of cheese properties (i. e. typical  
515 aromas, texture, flavors). In developed countries, cheeses are manufactured with pasteurized milk, and  
516 raw-milk cheese diffusion is limited, even in Europe, despite a derogative directive (92/46) of the EEC  
517 (Licitra, Caccamo, & Lortal, 2019). However, traditional and artisan raw-milk cheeses are produced  
518 worldwide and have an important economic role, and also a social and a cultural role. As plants are  
519 already traditionally used in cheese production methods for their aromatic properties, or in other food

520 products, plant additions in raw-milk in order to enhance the safety of cheeses respect the existing  
521 legislation (Food an Drug Administration - Food Ingredients & Packaging, 2019; Regulation EC  
522 N°1334/2008, 2008).

#### 523 **4.4. Commercial innovative systems with plant extracts**

524 Many edible plants are known for their antimicrobial and / or antioxidant potential. Nevertheless, only  
525 a few of them are actually marketed for their properties as food preservatives. This part presents some  
526 promising plant / plant extracts with a potential commercial application or already commercialized.  
527 Legume extracts, especially from the the Fabaceae family (e.g. cowpeas, soybean, peas) contain  
528 polyphenols or proteins known for their antimicrobial activity against foodborne pathogens like  
529 *L. monocytogenes* or *Salmonella* spp. (Pina-Pérez & Ferrús Pérez, 2018, Sitohy, Mahgoub, & Osman,  
530 2012) or antioxidant properties (Jayathilake et al., 2018). For example, esterified proteins from  
531 soybean and chickpea enhance raw buffalo milk conservation, even at room temperature (Osman,  
532 Mahgoub, El-Masry, Al-Gaby, & Sitohy, 2014, (M. Sitohy, Mahgoub, & Osman, 2011). Nevertheless,  
533 there are no preservatives based on legume extracts currently on the market.

534 In some Asians coutries, all bamboo parts (e.g. shoot skin, seeds, leaves) are largely used for industrial  
535 and domestic uses (notably as a traditional source of food). Furthermore, bamboo has been used in  
536 medicine since ancient times (Nirmala, Bisht, Bajwa, & Santosh, 2018). Studies revealed that bamboo  
537 contains various bioactive compounds especially antioxidant which can be beneficial to the  
538 consumer's health. Furthermore, the bamboo species *Phyllostachys pubescens* is known for it is  
539 antimicrobial activity against *Staphylococcus aureus* (Tanaka, Kim, Oda, Shimizu, & Kondo, 2011), and  
540 bamboo vinegar can be used to developped edible coating for pork chops conservation (Zhang, He,  
541 Kang, & Li, 2018). Bamboo properties are now exploited by the Take X society (Japan). Takex  
542 Fresh™ is a bamboo extract based food additive (alcohol formulation) that can be used instead of  
543 sodium hypochlorite (100 ppm) to wash foods like vegetables or fish in order to improve their storage  
544 life.

545 BioVia™ YM 10 (Danisco, Denmark) contains, notably, green tea extract, and mustard essential oil;  
546 it has been developed to control food spoilage through yeast and mold. Freshglow Co (USA) sells  
547 sheets of paper that have been infused with plant extract like Fenugreek and claims that these sheets  
548 can enhance fruit and vegetable conservation time at home from 2 to 4 folds.

549 Rosemary extracts for food preservation are commercially available, with, for example, the Santiox®  
550 (Santis, Morocco), a natural discolored and deodorized extract from rosemary leaves containing 15%  
551 carnosic acid, 1 to 4% carnosol and 1 to 6% rosmarinic acid which claims antioxidant efficiency with  
552 applications for milk and dairy products. Another commercial product : Guardox™ RA (Hunday, Belgium)  
553 is a rosemary extract that is rich in rosmarinic acid, with minimal odor and bitterness which  
554 was developed to delay lipid oxidation or rancidity on a wide range of products (e.g. cookies, soft  
555 drinks, cooked meat, ready to eat meals).

556 Hydroxytyrosol is a major constituent of olives that possesses antimicrobial and antioxidant activities  
557 (Robles-Almazan et al., 2018) without genotoxic effects (Kirkland, Edwards, Woehrle, & Beilstein,  
558 2015). It is currently commercialized by CREAGRI, Inc.® (USA) in Hidrox®. The antimicrobial and  
559 antioxidant properties of Hidrox® for food preservation is supported by literature (Soni, Burdock,  
560 Christian, Bitler, & Crea, 2006). In the same way, Citrox® (New Zealand) is a citrus extract based  
561 product that is on the market for the food industry. It has been demonstrated that Citrox® can affect  
562 foodborne bacteria growth in dairy based products (Tsiraki et al., 2018), (Tsiraki & Savvaidis, 2016).

## 563 **5. Conclusion**

564 Numerous plants are traditionally used in cheese making, for their flavouring properties as well as for  
565 their technological properties (e.g. milk clotting, packaging). Some of those plants contain peptides  
566 and/or secondary metabolites that exhibit antimicrobial properties against foodborne pathogens. In  
567 Mediterranean countries, dairy products, mainly raw milk, play an important role in the local  
568 economic sector, and in the diet of local consumers. However, the contamination level of raw milk  
569 with pathogenic strains might be enhanced by the lack of refrigeration or adequate transformation  
570 equipments. Thus, delaying microbial contamination of raw milk with plants traditionally associated

571 with dairy products might be part of the solution. Such solutions can be developed in all the  
572 Mediterranean countries while respecting regulations and economic considerations. Also, scientific  
573 studies must prove the antimicrobial effect against pathogenic and spoilage microorganisms, while  
574 preserving the technological flora implicated in milk clotting and ripening.

575

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582



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1084 **Figure caption**

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1086 **Figure 1: Potential of vegetal matter traditionally used in cheese processing and its pros and cons for raw milk cheese preservation**

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1090 **Table 1: Traditional cheese made with plant or plant extract (Sicard, 2018)**

PLANT	CHEESE	ORIGIN	MILK PROCESSING	RIPENING
<b>Addition of plants in cheese curd</b>				
<b>Cow milk</b>				
<i>Allium sativum</i> (garlic)	Gaperon	France	raw, butter	3 to 4 weeks
	Spinosien à l'ail		raw	8 months
	Boursin à l'ail, P'Ail		pasteurized	none
<i>Allium schoenoprasum</i> L (chives)	Boursin		pasteurized	none
<i>Allium ursinum</i> (wild/bear's garlic)	Tomme fermière à l'ail des ours		raw	2 months
	Tomme vaudoise à l'ail des ours	Switzerland	raw/thermized	2 months
<i>Brassica nigra/Sinapis nigra</i> (Mustard)	Mustard seed Gouda	Netherlands	raw/pasteurized	2 months
<i>Capsicum annuum</i> (paprika)	Mossa	France	pasteurized	none
<i>Cuminum cyminum</i> (cumin)	Altenburger Ziegenkäse	Deutschland	raw*	4 weeks
	Cumin seed Gouda	Netherlands	raw/pasteurized	2 months
	Munster au cumin		raw/pasteurized	2-3 months
	Tomme vaudoise au cumin		raw/thermized	2 months
<i>Piper nigrum</i> (pepper)	Boursin au poivre, Mossa	France	pasteurized	none
	Gaperon		raw, butter	3 to 4 weeks
	Holunderkas		pasteurized	3 weeks
<i>Sambucus nigra</i> (elderberry flowers)			raw	2 months
<i>Urtica dioica</i> (nettle)	Tomme fermière aux orties,		raw	8 months
	spinosen aux orties		raw	8 months
<b>Sheep milk</b>				
<i>Capsicum annuum</i> (smoked paprika)	The Black Sheep	New Zealand	pasteurized	10 months
<i>Piper nigrum</i> (pepper)	Piacentinu ennese	Italy	raw/pasteurized	3 to 12 months
<i>Thymus vulgaris</i> (thyme)	Brebichou au Thym (Thyme EO)	France	raw	2 weeks

Cheeses with plant extract addition for coagulation				
<b>Cow milk</b>				
<i>Calotropis procera</i> (apple of Sodom)	Peulh cheese	Benin	raw	12 days
<i>Gallium verum</i> (lady's bedstraw)	Gloucester cheese	U.K.	raw/pasteurized	4-12 weeks
<b>Goat milk</b>				
<i>Ficus carica sylvestris</i> (caprifig)	Ficu, Cacioricotta	Italia	pasteurized	35 days
<b>Sheep milk</b>				
<i>Cynara spp.</i> (wild cardoon flower)	Serra da Estrala	Portugal	raw	30 days
	Serpa		2 years	
	Azeitao		20 days	
	Nisa		45 days	
	Castelo Branco	Spain	raw	8 days
	Torta del Casar			60 days
	La Serena			60 days
	Los Pedroches			60-80 days
Addition of plants on the surface of cheese				
<b>Cow milk</b>				
<i>Allium sativum</i> (garlic)	Cabris* (along with shallots)	France	raw/pasteurized	2 weeks
<i>Allium schoenoprasum</i> L (chives)	Boulette de la Pierre-qui-Vire		raw	2-3 weeks
<i>Brassica nigra/Sinapis nigra</i> (Mustard)	Délice de Pommard		pasteurized	none
<i>Capsicum annuum</i> (paprika)	Boeren-Leidse met sleutels	Netherlands	raw	3 to 12 months
	Ridge line	U.S.A	raw	2 months
	Boulette d'Avesnes,	France	pasteurized	None
	Figurette		raw	none
<i>Piper nigrum</i> (pepper)	Vergalor		raw	4 months
	Colombier de Sivry		raw	4 weeks
	Rigotte au poivre		pasteurized	2-3 weeks
<i>Rosmarinus officinalis</i> (rosemary)	Colombier de Sivry		raw	4 weeks
<i>Salvia sclarea/Salvia officinalis</i> (sage)	Colombier de Sivry		raw	4 weeks

<b>Goat milk</b>					
<i>Cuminum cyminum</i> (cumin)	Figuette, Lou Pèbre		raw		None
<i>Piper nigrum</i> (pepper)	Chabis de Gâtine au poivre, Lou Pèbre		raw		None
<i>Rosmarinus officinalis</i> (rosemary)	Bouyguette		raw		2-3 weeks
<i>Thymus vulgaris</i> (thyme)	Rovethym		raw		3 weeks
<i>Rosmarinus officinalis</i> (rosemary)	Brin d'amour (+Juniper Berries and savory)		raw /whey		30-45 days
<b>Sheep milk</b>					
<i>Mentha viridis</i> (spearmint)	Halloumi	Cyprus	pasteurized		40 days , in brine
<i>Thymus vulgaris</i> (thyme)	Saveur du Maquis (+ oregano, savory, majoram)	France	pasteurized		None
<b>Cheeses with tree leave</b>					
<b>Cow milk</b>					
<i>Acer pseudoplatanus</i> (sycamore maple leaves)	Valdeón*	Spain	raw/pasteurized		2-3 months
<i>Allium ursinum</i> (wild/bear's garlic leaves)	Wild Garlic Yarg	U.K.	pasteurized		5 weeks
<i>Castanea sativa</i> (chestnut leaves)	Feuille de Dreux	France	pasteurized		2 weeks
	Rogue river blue	U.S.A.	raw		8 months
<i>Urtica dioica</i> (nettle leaves)	Cornish Yarg	U.K.	pasteurized		5 weeks
<i>Vitis vinifera</i> (grape leaves)	Rogue river blue	U.S.A.	raw		8 months
<b>Goat milk</b>					
<i>Acer pseudoplatanus</i> (sycamore maple leaves)	River's Edge Up in Smoke	U.S.A.	pasteurized		1 week
<i>Castanea sativa</i> (chestnut leaves)	Banon,	France	raw		15 days
	Mothais-sur-feuille		raw		4 weeks
	Cabrales (trad.)	Spain	Raw*		2 months
<i>Ficus carica sylvestris</i> (caprifig)	Ficu, Ubriaco*	Italy	pasteurized		2-12 months
<i>Platanus xhispanica</i> (plane tree leaves)	Couhé verac	France	raw		3-4 weeks
<i>Prunus avium</i> (cherry leaves)	Robiola La Rossa	Italy	raw		10-15 days
<i>Piper auritum</i> (hoja santa leaves)	Hoja Santa	U.S.A.	pasteurized		1-6 months
<b>Sheep milk</b>					
<i>Juglans regia</i> L. (walnut leaves)	Pecorino Foglie di	Italy	raw		3 months



Noce				
<i>Olea europea</i> (oliv leaves)	Pecorino "L'Ulivastro", Foglie di ulivo, L'Ulivo	Italy	pasteurized	3 months
1091	*other milks also used			

**Table 2: Biological activities of plants traditionally used for cheese-making**

Species	Part of the plant and/or extraction method	Biological activity	Model / Food	Reference
<i>Allium ascalonicum</i> (shallot)	Essential oil	Antimicrobial activity against <i>EC</i> O157:H7	Iranian white brined cheese	Ehsani & Mahmoudi, 2012
<i>Allium ursinum</i> (Wild garlic)	Powder of leaves extracted with ethanol, water, methanol	Antioxidant properties, antimicrobial activity against <i>Salmonella enteritidis</i> , <i>EC</i> , <i>Proteus mirabilis</i> and <i>Enterococcus faecalis</i>	<i>in vitro</i> tests	Pavlović et al., 2017
<i>Allium roseum</i> (rosy garlic)	Dried methanol extract of fresh aerial parts and bulbs	Antioxydant properties, anti <i>Candida sp.</i> activity Antimicrobial activity against 13 Gram-negative strains including <i>ST</i> , <i>S. anatum</i> , <i>S. enteridis</i> and <i>EC</i> and three Gram-positive strains including <i>SA</i>	<i>in vitro</i> tests	Snoussi et al., 2016
<i>Allium sativum</i> (garlic)	Aqueous extract	Antimicrobial activity against <i>SE</i> and <i>Salmonella spp.</i>	<i>in vitro</i> tests	El-Azzouny, El-Demerdash, Seadawy, & Abou-Khadra, 2018
	Ethanol extracts of fresh and dried plants	Antioxydant properties, antimicrobial activity against <i>ST</i> , <i>EC</i> , <i>SA</i> and <i>Enterococcus faecalis</i>	Cottage cheese	Josipović et al., 2015
	Ethanol extract	Antimicrobial activity against <i>ST</i> , <i>EC</i> , <i>SA</i> and <i>LM</i>	Dairy based media	Tayel et al., 2015
<i>Bixa orellana</i> (achiote tree)	Commercial Annato extract	Antimicrobial activity against numerous food borne pathogens	<i>in vitro</i> tests	Galindo-Cuspinera et al., 2003
<i>Castanea sativa</i> (chestnut)	Crude methanol extract of the ground leaves	Quorum sensing inhibitory activity against <i>SA</i>	<i>in vitro</i> tests.	Quave et al., 2015
	Aqueous extract	Antimicrobial activity against <i>SA</i> , <i>Streptococcus pneumonia</i> , <i>EC</i> , <i>Klebsiella pneumoniae</i> , <i>Proteus mirabilis</i> and <i>Pseudomonas aeruginosa</i>	<i>in vitro</i> tests	Dashtdar, shirazi, & Khan, 2013
	Aqueous sulfuric acid extract (pH = 3) of leaves	Antimicrobial activity against <i>SA</i> , <i>Proteus vulgaris</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>EC</i> , <i>ST</i> and <i>Enterobacter aerogens</i>	<i>in vitro</i> tests	Basile et al., 2000
<i>Cedrus deodara</i> (pine)	Aqueous extract of needles	Antimicrobial activity: lower values for total plate count, psychrophilic count and yeast and mould count	Cheese	Mahajan et al., 2016
<i>Crocus sativus</i> (saffron)	Saffron dried stigmas thread like parts of the flower extract	Antioxydant properties, antimicrobial activity against coliform and <i>enterococci</i> groups	Fresh ovine cheese	Aktypis et al., 2018
<i>Cynara</i>	Solutions obtained from dry ethanolic	Antioxidant, antimicrobial activity against <i>ST</i> , <i>EC</i> ,	<i>in vitro</i> tests	Kukić et al., 2008

<i>cardunculus</i> (cardoon)	Extract of leaf submitted to diverse liquid-liquid partition	<i>Bacillus subtilis</i> , <b>SE</b> , <b>SA</b> , and eight fungi strains		
	Lyophilized aqueous extract from flowers	No antibacterial effect	ewes' milk cheese	Galán et al., 2012
	Leaves, flowers and seeds Solvent: hexane, acetone, methanol, water	Antioxydant properties, antimicrobial activity	<i>in vitro</i> tests	Falleh et al., 2008
<i>Cuminum cyminum</i> (cumin)	Essential oil	Antimicrobial activity against <b>SA</b> , no effect against <i>Lactobacillus acidophilus</i>	Iranian white brined cheese	Sadeghi et al., 2013
	Seed oil	Antimicrobial activity against <i>Salmonella enterides</i> and <b>EC</b>	Domiaty cheese	Mahgoub et al., 2013; Hassanien et al., 2014
	Ethanol extract from seeds	Antimicrobial activity against <b>SA</b>	<i>in vitro</i> tests	Mostafa et al., 2018
<i>Inula britannica</i> (British yellowhead)	Lyophilized aqueous extract from flowers	Antioxidant properties	Cheddar type cheese	N.-K. Lee et al., 2016
<i>Matricaria recutita</i> (chamomille)	Dried chamomile and decoction of dried chamomile	Antioxidant and antifungi properties Antimicrobial activity against <b>SA</b> , <i>Bacillus cereus</i> , <b>LM</b> , <i>Pseudomonas aeruginosa</i> , <b>EC</b> , <i>Enterobacter. cloacae</i> and <b>ST</b>	Cottage cheese	Caleja et al., 2015
Olive	Commercial olive leaf extract	Antimicrobial activity against numerous microorganisms among which <b>LM</b> , <b>SA</b> , <b>EC</b> and <i>Salmonella sp.</i>	<i>in vitro</i> tests	Sudjana et al., 2009
	Polyphenol extract from olive oil by-product	Antimicrobial activity against <i>Pseudomonas fluorescens</i> and <i>Enterobacteriaceae</i>	Fior di latte cheese	(Roila et al., 2019)
<i>Origanum vulgare</i> (oregano)	Essential oil	Antimicrobial activity	fresh goat cheese	Zantar et al., 2014
	Essential oil from aerial parts	Antimicrobial activity against <i>Enterobacter sp.</i> but no effect on lactic starter cultures	Traditional Argentinean cheese	Marcial et al., 2016
	Essential oil	Antimicrobial activity against <b>EC</b> O157:H7 and <b>LM</b>	Feta cheese	Govariz et al., 2011
	Ethanol extract from leaves	Antimicrobial activity against <b>ST</b> , <b>EC</b> O157:H7, <b>SA</b> and <b>LM</b>	Dairy based media	Tayel et al., 2015
	Essential oil	Antimicrobial activity against <b>EC</b> O157:H7	Fresh cheese	(Diniz-Silva et al., 2019)
	Essential oil	Antimicrobial activity against <b>EC</b> O157:H7	Minas frescal cheese	(Diniz-Silva et al., 2019)

<i>Ocimum basilicum</i> (basil)	Leaves in dehydrated form or decoction	Antioxydant properties, antimicrobial activity against eight bacteria and eight fungi strains	“Serra da Estrela” cheese	Carocho et al., 2016
<i>Pimpinella anisum</i> (anise)	Essential oils	Antimicrobial activity against <i>EC</i> O157:H7	Iranian white brined cheese	Ehsani & Mahmoudi, 2012
<i>Rosmarinus officinalis</i> (rosemary)	Rosemary extract (Type HT-125)	Antioxydant properties	cow and soy milk enriched with fish oil	Qiu 2018
	Aqueous ethanolic and methanolic extracts on aerial part	Antimicrobial activity against five Gram-positive strains including <i>SA</i> , <i>SE</i> and <i>LM</i> and seven Gram-negative strains	<i>in vitro</i> tests	Kozłowska, Laudy, Przybył, Ziarno, & Majewska, 2015
	Ethanol and acetone extract	Antioxydant, antimicrobial activity against <i>ST</i> , <i>EC</i> , <i>SA</i> and <i>LM</i>	Cottage Cheese	Josipović et al., 2015
	Essential oil	Antimicrobial activity against <i>EC</i> O157:H7	Fresh cheese	(Diniz-Silva et al., 2019)
	Essential oil	Antimicrobial activity against <i>EC</i> O157:H7	Minas frescal cheese	(Diniz-Silva et al., 2019)
<i>Salvia officinalis</i> (sage)	Aerial part	Antimicrobial activity against five Gram-positive strains including <i>SA</i> , <i>SE</i> and <i>LM</i> and four Gram-negative strains	<i>in vitro</i> tests	Kozłowska, Laudy, Przybył, Ziarno, & Majewska, 2015
	Aqueous ethanolic and methanolic extracts			
	Ethanol extract from leaves	Antimicrobial activity against <i>ST</i> , <i>EC</i> O157:H7, <i>SA</i> and <i>LM</i>	Dairy based media	Tayel et al., 2015
<i>Syzygium aromaticum</i> (clove)	Extracted with a mixture of ethanol/water (4:1)	Antioxydant, antimicrobial activity against <i>Salmonella enterica</i> , <i>LM</i> , <i>SA</i>	cheese	Shan et al., 2011
<i>Thymus vulgaris</i> (thyme)	Essential oil	Antimicrobial activity	fresh goat cheese	Zantar et al., 2014
	Essential oil	Antimicrobial activity against <i>EC</i> O157:H7 and <i>LM</i>	Feta cheese	Govaris et al., 2011
	Essential oil	Antimicrobial activity against <i>SA</i> , <i>LM</i> and mesophilic starter	Cheese mimicking models	de Carvalho et al., 2015
	Aerial part	Antimicrobial activity against four Gram-positive strains including <i>SA</i> and <i>SE</i> , and six Gram-negative strains	<i>in vitro</i> tests	Kozłowska, Laudy, Przybył, Ziarno, & Majewska, 2015
	Aqueous ethanolic and methanolic extracts			
	Leaves	Antimicrobial effect against <i>SA</i> and <i>P. aeruginosa</i> .	<i>in vitro</i> tests	Mostafa et al., 2018
	Ethanolic extract			

<i>Thymus algeriensis</i> (thyme)	Essential oil	Antimicrobial activity against <b>SA</b> , <b>ST</b> , <i>Enterobacter cloacae</i> , <b>EC</b> , <b>LM</b> , <i>Pseudomonas aeruginosa</i> , <i>Micrococcus flavus</i> , <i>Bacillus cereus</i>	Soft cheese	(Bukvicki et al., 2018)
<i>Thymus mastichina</i> (thyme)	Ethanollic and aqueous extract	Antimicrobial activity	Cheese surfaces and ripening chamber	(F. Carvalho et al., 2018)
<i>Urtica doica</i> (nettles)	Water extract	Antimicrobial activity	Whey protein coating for preservation of rainbow trout fillets	Alp Erbay et al., 2017 Alp & Aksu, 2010
<i>Vitis vinifera</i> (grape vine)	Grape seeds from Greece (two varieties). Powder, extracted with hexane	Antioxidant properties, did not affect <i>Lactobacilli</i> counts	Full-fat and non-fat yoghurts	Chouchouli et al., 2013
	Grape juice and skin and seed extracts	Antimicrobial activity against <b>LM</b>	<i>in vitro</i> tests	Rhodes et al., 2006

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*EC*: *Escherichia coli*, *ST*: *Salmonella Typhimurium*, *SA*: *Staphylococcus aureus*, *SE*: *Staphylococcus epidermidis*, *LI*: *Listeria innocua*, *LM*: *Listeria monocytogenes*

**Table 3: Plant bioactive compounds families with potential use as food preservatives**

examples	Pathogen targeted	Source	Ref
<b>Alkaloids</b>			
Berberine	Inhibition of <i>SE</i> biofilm formation. Antifungal against four <i>Candida</i> species: MIC = 16-128 $\mu\text{g mL}^{-1}$	<i>Coptidis rhizoma</i>	Wang et al., 2009
Piperine	<i>EC</i> , <i>Klebsiella pneumonia</i> , <i>Salmonella enterica</i> , <i>SA</i> , <i>Enterococcus faecalis</i> : MIC = 625 $\mu\text{g.mL}^{-1}$ and <i>SE</i> , <i>Staphylococcus xylosus</i> , <i>Bacillus subtilis</i> : MIC = 312.5 $\mu\text{g.mL}^{-1}$	<i>Piper nigrum</i>	Zarai, Boujelbene, Ben Salem, Gargouri, & Sayari, 2013
<b>No-flavonoid phenolics</b>			
<b>Cinnamic acid</b>	Cinnamic acid	<i>ST</i> and <i>EC</i> : MIC = 7.5 and 5 mM respectively	<i>Arabidopsis thaliana</i> Olasupo, Fitzgerald, Gasson, & Narbad, 2003
<b>Cinnamic acid</b>	Ferulic acid	<i>EC</i> , <i>Pseudomonas aeruginosa</i> , <i>SA</i> , <i>LM</i> : MIC = 0.1, 0.1, 1.1 and 1.25 $\text{mg.mL}^{-1}$ respectively	Borges et al., 2013
<b>Lignans</b>	Styryl juponoside A	<i>Candida albicans</i> , <i>Trichosporon beigeli</i> , <i>Malassezia furfur</i> : MIC = 20 $\mu\text{g.mL}^{-1}$ for the three strains	Park et al., 2010
<b>Phenyl propanoid</b>	Eugenol	<i>ST</i> and <i>EC</i> : MIC = 3 and 2.5 mM respectively	<i>Solanum tuberosum</i> (potato) Olasupo et al., 2003
<b>Benzoic acid</b>	Gallic acid	<i>EC</i> , <i>Pseudomonas aeruginosa</i> , <i>LM</i> , <i>ST</i> and <i>SA</i> : MIC = 1.5, 0.5, 2, 2;5 and 1.25 $\text{mg.mL}^{-1}$ respectively	<i>Lawsonia inermis</i> (Henna) Borges, Ferreira, Saavedra, & Simões, 2013; Chanwitheesuk, Teerawutgulrag, Kilburn, & Rakariyatham, 2007
<b>Benzoic acid</b>	Chlorogenic acid	<i>Streptococcus pneumoniae</i> , <i>SA</i> , <i>Bacillus subtilis</i> , <i>EC</i> , <i>Shigella dysenteriae</i> and <i>ST</i> , MIC = 20, 40, 40, 80, 20 and 40 $\mu\text{g.mL}^{-1}$ respectively	prune, aromatic herbs Lou, Wang, Zhu, Ma, & Wang, 2011
<b>Prenylated benzyl</b>	Erybraedin A	five <i>Streptococcus</i> strains: MIC = 0.78-1.56 $\mu\text{g.mL}^{-1}$ , <i>SA</i> : MIC = 3.13 $\mu\text{g.mL}^{-1}$ and seven drug-resistant strains of <i>SA</i> : MIC = 0.78-6.25 $\mu\text{g.mL}^{-1}$	<i>Erythrina subumbrans</i> Rukachaisirikul et al., 2007
<b>Stilbene</b>	E-resveratrol	Antioxidant and antimicrobial against <i>Penicillium expansum</i> and <i>Aspergillus niger</i>	(blackberries, grape skin) Filip, 2003; Stojanović, Sprinz, & Brede, 2001
<b>Stilbene</b>	Vitisin B	<i>Pseudomonas aeruginosa</i> and <i>EC</i> : biofilm formation inhibition	<i>Paeonia lactiflora</i> (seed) Lee, Kim, Ryu, Cho, & Lee, 2014

Flavonoïd				
<b>flavanol</b>	Epigallocatechin-3-O-gallate	<i>SA</i> , <i>Salmonella</i> (ATCC 9270 and ATCC 13314), <i>EC</i> and <i>Vibrio</i> (ATCC 17802): MIC = 98, 308, 602 and 68 µg.mL <sup>-1</sup> respectively		Taguri, Tanaka, & Kouno, 2004
<b>flavonol</b>	Petalostemumol	<i>Candida albicans</i> , <i>Cryptococcus neoformans</i> , <i>Mycobacterium intracellulare</i> and <i>EC</i> : MIC = 12.5, 25, 6.25, 6.25 and 0.78 µg.mL <sup>-1</sup> respectively	Purple prairie clover	Hufford et al., 1993
Peptides				
<b>Defensin</b>	Thionins	<i>LM</i> , <i>LI</i> , <i>Listeria ivanovii</i> : MIC = 2, 2, 5 µg.mL <sup>-1</sup> respectively	<i>Triticum aestivum</i> (wheat)	Lopez-Solanilla, Gonzalez-Zorn, Novella, Vazquez-Boland, & Rodriguez-Palenzuela, 2003
<b>Cyclotide</b>	Cycloviolacin O2	<i>EC</i> , <i>Salmonella enterica</i> , MIC = 2.2, 8.75 µM respectively	<i>Viola odorata</i> (violet)	Pranting, Loov, Burman, Goransson, & Andersson, 2010
<b>Snakin</b>		<i>LM</i> , <i>LI</i> , <i>Listeria ivanovii</i> : MIC = 10 µg.mL <sup>-1</sup> for all	<i>Impatiens balsamina</i> (balsam)	Lopez-Solanilla et al., 2003
Terpenoïds				
<b>monoterpene</b>	Thymol	<i>ST</i> and <i>EC</i> : MIC = 1 and 1.2 mM respectively, then <i>SA</i> , <i>LI</i> , <i>EC</i> , <i>Aspergillus niger</i> : MIC = 250 ppm for all) and <i>Saccharomyces cerevisiae</i> : MIC = 125 ppm	<i>Oldenlandia affinis</i>	Guarda, Rubilar, Miltz, & Galotto, 2011; Olasupo et al., 2003
<b>monoterpene</b>	Carvacrol	<i>ST</i> and <i>EC</i> : MIC = 1 and 1.5 mM respectively and <i>SA</i> , <i>LI</i> , <i>Aspergillus niger</i> : MIC = 225 ppm for all and <i>Saccharomyces cerevisiae</i> : MIC = 75 ppm	<i>Hordeum vulgare</i> (barley)	Guarda et al., 2011; Olasupo et al., 2003

*EC*: *Escherichia coli*, *ST*: *Salmonella Typhimurium*, *SA*: *Staphylococcus aureus*, *SE*: *Staphylococcus epidermidis*, *LI*: *Listeria innocua*, *LM*: *Listeria monocytogenes*

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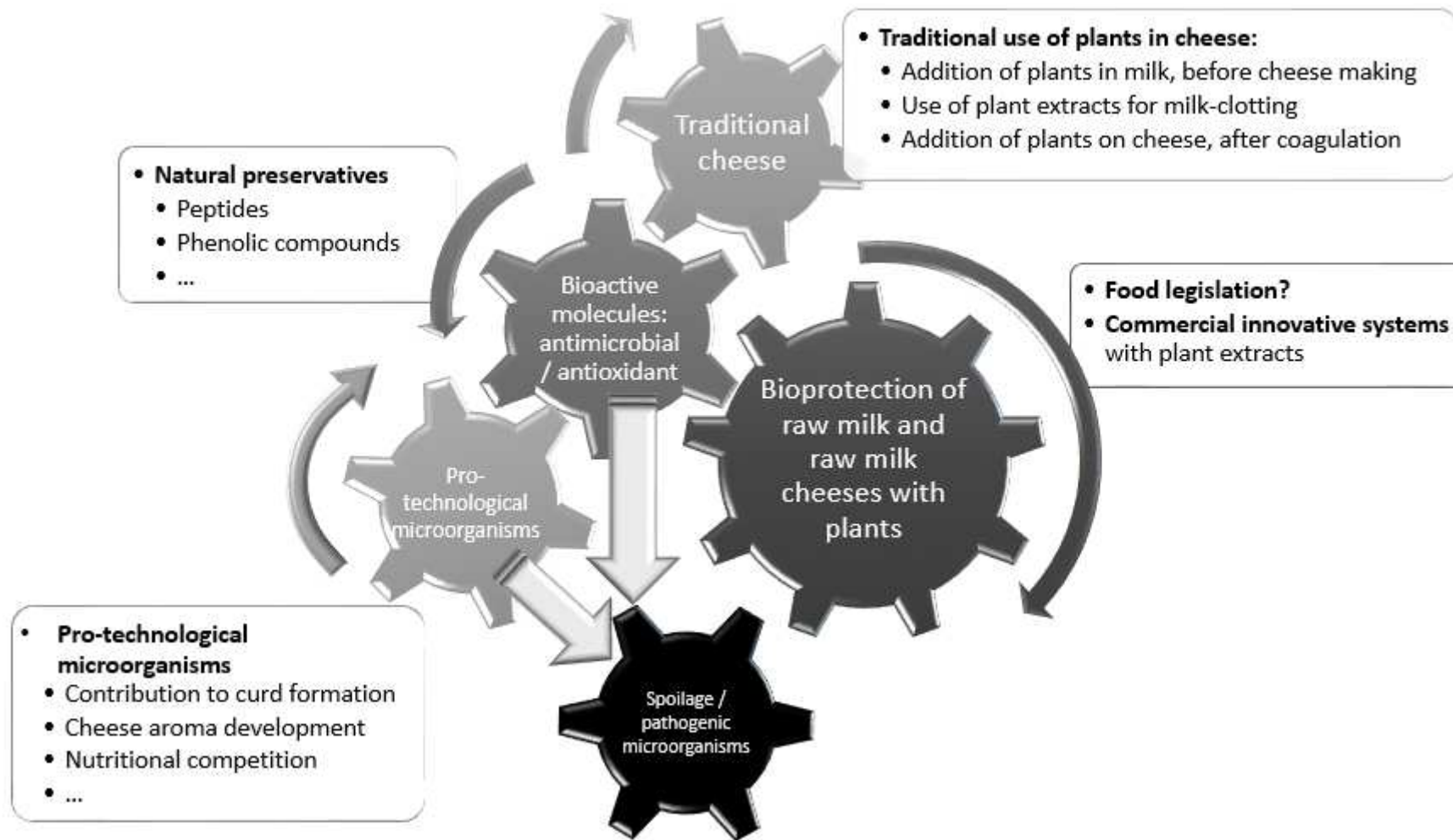


Figure 1



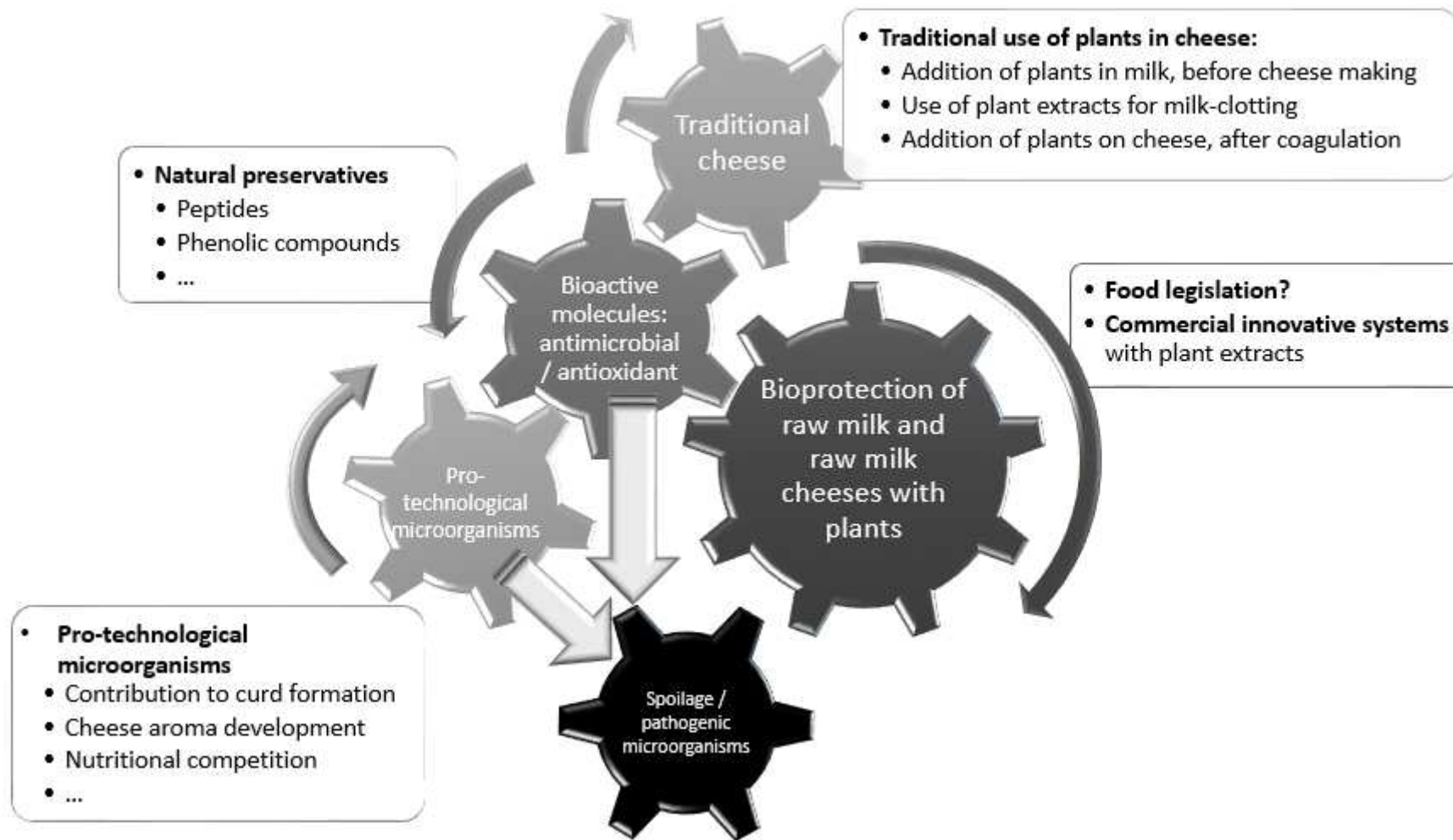


Figure 1