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1 **Sorption kinetic of aroma compounds by edible bio-based films from**
2 **marine-by product macromolecules: effect of relative humidity conditions**

3

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22

23

24 **Abstract**

25 Edible films based on gelatin and chitosan have high gas and aroma barrier properties. This
26 study focused on their capability to sorbed/retain aroma compounds (1-hexanal, 2-hexen-1-ol,
27 1-hexanol, 3-hexanone and phenol) at three relative humidity level ($\leq 2\%$, 53% or 84% RH).
28 Whatever the relative humidity condition, the order of sorption is keton (3-hexanone)
29 <aldehyde (1-hexanal) <aliphatic alcohols (2-hexen-1-ol and 1-hexanol) < phenol. This order
30 could be related to the intrinsic chemical properties of aroma compounds. The increase in
31 moisture enhanced the sorption at the highest RH for all the aroma compounds. However, a
32 competition between water and aliphatic alcohols is observed at 53%RH. All compounds
33 have an ideal sorption behaviour (logarithmic increase) except 1-hexanal. The sorption of 1-
34 hexanal, 1-hexanol, 2-hexen-1-ol and 3-hexanone induced an antiplasticization of the
35 network by increasing the film Tg by more than 5°C. On the contrary, phenol was an efficient
36 plasticizer at least as high as moisture.

37

38 Keywords: active edible films; aroma sorption; structure properties; glass transition;
39 plasticization; antiplasticization.

40

41 **1. Introduction**

42 Food is required to satisfy the biological need for a source of nutrition but its quality is easily
43 diminished by the harmful transport of aroma compounds and oxygen. Indeed, it is the flavor
44 and aroma of a food that provide the impetus for its consumption. In fact, a large segment of
45 commercial manufacturing deals with the production of packaging that extends the shelf life
46 of food by controlling flavor and aroma transport (Miller & Krochta, 1997).

47 The first role of the packaging material is to provide an adequate shelf life and product
48 quality, but it is desirable that packaging also participates in the overall flavor management of
49 the packaged food, as flavor being an underlying factor in the consumer acceptability of all
50 food products. Sorption of aroma compounds in packaging polymers is of interest as it might
51 cause an imbalance in the food's flavour profile thereby deteriorating the sensorial quality of
52 the packaged product during storage (Dury-Brun, Chalier, Desobry, & Voilley, 2007). These
53 causes change both the intensity and characteristics of the food flavors owing to their
54 absorption by the packaging material. This phenomenon is commonly referred to as
55 "scalping" (Sajilata, Savitha, Singhal, & Kanetkar, 2007). Therefore, aroma scalping or
56 sorption became an important parameter for polymer film selection (Leelaphiwat, Auras,
57 Harte, Ong, & Chonhenchob, 2016).

58 This subject is mainly concerned with plastic polymers and was highly reviewed (Caner,
59 2011). Flavour scalping by plastic films in contact with foods, particularly polyolefin, is well-
60 documented in the literature (Sajilata, Savitha, Singhal, & Kanetkar, 2007; Willige, Linssen,
61 & Voragen, 2000).

62 These plastic films allow an important and selective reduction of non-condensable gas and
63 water vapor exchanges such as that obtained with high barrier polymers, and they are usually
64 employed as interior linings for the heat sealing in contact with foods. Nevertheless, most of
65 them have a strong affinity, due to their olefinic structure, toward hydrophobic volatile

66 compounds, such as aroma compounds (Quezada Gallo, Debeaufort, & Voilley, 1999).
67 Moreover, plastic materials cannot be used to protect all kinds of foods: for example, to
68 separate two different parts in a heterogeneous product such as a pie or a pizza (Kester &
69 Fennema, 1989). In contrast, hydrophilic polymers can be expected to present low affinities
70 for apolar compounds and hence a reduced tendency to cause flavour scalping (Balaguer,
71 Gavara, & Hernández-Muñoz, 2012).

72 Hydrophilic films obtained from polysaccharides and proteins are attracting considerable
73 interest in food packaging applications. These biopolymers, as they are extracted directly
74 from renewable resources, are sustainable and their biodegradability is in custody with
75 environmental protection (Balaguer, Gavara, & Hernández-Muñoz, 2012). However, major
76 drawback of these polymeric films include their solubility in water and the lack of
77 mechanical strength, especially under wet environments, which limits their application as
78 packaging materials (Woerdeman, Veraverbeke, Parnas, Johnson, Delcour, Verpoest, et al.,
79 2004). But, when packaging is required for just short periods (film wrappings, laminated
80 papers, etc.), hydrophilic biopolymers present attractive properties such as good aroma and
81 oxygen barrier properties at low and intermediate humidities (Bordenave, Grelier, Pichavant,
82 & Coma, 2007). Furthermore, these polymers can also be processed, as a fine layer, into self-
83 standing plastics for food packaging applications in order to reduce flavour scalping during
84 storage but also as carrier of active compounds.

85 There is, however, little information in the literature concerning the flavour scalping by
86 hydrophilic hydrocolloids films in contact with foods (Quezada Gallo, Debeaufort, &
87 Voilley, 1999) compared the methyl ketones (aroma) sorption by both the low density
88 polyethylene (plastic) and the methylcellulose (edible films). At concentrations of methyl
89 ketones close to those in foodstuffs, edible films have better barrier properties against aromas
90 than low density polyethylene (LDPE) plastic films have. Indeed, these authors displayed that

91 permeability to aroma compounds of low density polyethylene is from 10 to 500 times lower
92 than that of methylcellulose-based films because of its apolarity which emphasized aroma
93 sorption by LDPE. The application of edible coatings or films to improve plastic packaging
94 properties could therefore be envisaged. Balaguer, Gavara, & Hernández-Muñoz.(2012)
95 reported that chitosan and gliadin films have very low capacities for the sorption of volatile
96 compounds, and these capacities are influenced by the nature of the sorbed compound, the
97 environmental relative humidity and the presence of glycerol as a plasticizer in the polymeric
98 matrix. Given the low levels of interaction observed with the volatiles, hydrophilic chitosan
99 and gliadin films are of potential interest for the packaging of foods in which aroma is one of
100 the most important quality attributes.

101 On one side, chitosan is nontoxic, biodegradable, biofunctional, biocompatible semi-natural
102 polysaccharide produced industrially by the chemical deacetylation of chitin, a major
103 component of crustacean shells (crab and shrimp) and the second most abundant biopolymer
104 present in nature after cellulose. Chitosan is soluble in aqueous acidic solutions becoming a
105 cationic polyelectrolyte with antimicrobial properties (Moradi, Tajik, Razavi Rohani,
106 Oromiehie, Malekinejad, Aliakbarlu, et al., 2012). In view of these properties, chitosan films
107 have been used as a packaging material for the quality preservation of a variety of foods
108 (Park & Zhao, 2004). On the other side, gelatin is one of the multipurpose biomaterials
109 obtained by the controlled hydrolysis of the insoluble fibrous collagen present in the bones
110 and skin, generated as waste during animal slaughtering and fish processing. Both gelatin and
111 chitosan were envisaged as biopolymers of potential interest for food-contact packaging
112 materials because of their favourable film-forming and high oxygen barrier properties
113 (Benbettaieb, Tanner, Cayot, Karbowiak, & Debeaufort, 2018; Hoque, Benjakul, & Prodpran,
114 2011; Jridi, Sellimi, Lassoued, Beltaief, Souissi, Mora, et al., 2017). Although the gas and
115 water vapour barrier properties of these polymers have been extensively analysed, very lack

116 information has been reported in the literature on their interaction with food aroma
117 compounds.

118 Several factors influence the absorption of aroma compounds into polymeric packaging
119 materials: the polymer **3D network** structure, the chemical composition, morphology and
120 crystallinity of the polymer, the material cohesion maintained by weak energy bonds, or the
121 polymer's glass transition temperature (Salazar, Domenek, & Ducruet, 2014). In addition, the
122 physicochemical characteristics of aroma compounds (polarity, shape and size...), their
123 chemical composition and functional groups, concentration and mixture, are important
124 criteria that influence their sorption. Furthermore, external factors like storage time, relative
125 humidity, temperature and pH can also affect solubility of aroma compounds in a polymer or
126 biopolymer (Fayoux, Seuvre, & Voilley, 1997).

127 The aim of the current work was to study the sorption behaviour of different aroma
128 compounds into chitosan-gelatin films. For this purpose five volatile molecules: 1-hexanal, 1-
129 hexanol, 2-hexen-1-ol, phenol and 3-hexanone, having 6 carbon atoms were selected to
130 compare the effect of different chemical functions (aldehyde, alcohols, ketones) and different
131 structures (aliphatic or ring) in a limited range of molecular weight. These aroma compounds
132 were chosen to represent the main chemical families of volatile compounds present in fruits,
133 vegetables and dairy products. Due to the hydrophilic nature of these biopolymers, the
134 kinetics of aroma sorption was assayed at room temperature at different relative humidities.
135 As the film structure can be easily modified by the aroma compound sorption, the thermal
136 properties of films were also analysed at the end of the kinetics of aroma sorption and
137 discussed regardless the sorbed quantity and the structure and physicochemical properties of
138 the aroma compounds.

139 **2. Materials and reagents**

140 A commercial food grade chitosan (France Chitine) and a commercial grade fish gelatin
141 (Rousselot 200 FG), were the filmogenic biopolymer used for the film matrix. The chitosan
142 (CS) characteristics are: MW=165 kDa, low viscosity, 85% deacetylation degree), and those
143 of fish gelatine (FG) are: 180 Bloom degree, a 4 mPas viscosity at 45⁰C and for a
144 concentration of 6.67% in water and at pH=5.4.

145 Glacial acetic acid (Sigma, 99.85% purity) was used to prepare the solvent for chitosan and to
146 improve its dispersion. A 1% acetic acid (Sigma, 99.85% purity) aqueous solution allowed
147 the dispersion and solubilisation of the chitosan powder. Anhydrous glycerol (GLY, Fluka
148 Chemical, 98% purity, Germany) was used to plasticize and thus improve the mechanical
149 properties of the films.

150 Five aroma compounds having 6 carbon atoms were selected to compare the effect of
151 different chemical functions (alcohols, ketons and aldehyde) and different structures
152 (aliphatic or ring) in a limited range of molecular weight on the sorption behaviour.

153 The selected aroma compounds are given hereafter which the physical and chemical
154 properties are detailed in Table 1.

- 155 • 1-Hexanol (Sigma-Aldrich, purity > 99%) a sweet organic alcohol and a component
156 of the odour of freshly mown grass mainly used in the perfume industry,
- 157 • 2-Hexen-1-ol, (Sigma-Aldrich, purity > 95%) it is a compound responsible for the
158 flavouring of cereal products and some candies,
- 159 • Phenol (Sigma-Aldrich, purity > 99.5%) a very sweet and acrid flavour used in
160 antiseptics and the cosmetic industry,
- 161 • 3-Hexanone, (Sigma-Aldrich, purity >97%) a sharp odour, previously used as paint
162 thinner and used in dissolving oils and waxes.

163 • 1-Hexanal (Sigma-Aldrich, 98% purity) a grass green odour which is responsible for
164 the slight fruity flavour of foods.

165

166 The relative humidities used for equilibration of films prior experiments and for fixing the
167 RH during experiments (kinetics of sorption) were :

168 - dried silicagel (WVR France, 98% purity) for $RH \leq 2\%$ at $25^{\circ}C$,

169 - NaBr (Sigma-Aldrich, 99% purity) saturated salt solution for 53% RH at $25^{\circ}C$

170 - And KCl (Sigma-Aldrich, 99% purity) saturated salt solution for 84% RH at $25^{\circ}C$

171 Silicagel was dried at $105^{\circ}C$ for one night and saturated solutions freshly prepared prior
172 using.

173

174 3. Methods

175 3.1. Film Preparation

176 A chitosan solution was prepared by dispersing 20g of the chitosan powder in 1L of the 1%
177 acetic acid aqueous solution. The solution was homogenised at 1200rpm with a high shear
178 homogeniser Ultra Turrax (RW16 basic IKA – WERKE). 2.22g of glycerol (10% w/w dry
179 matter polymer) was then added to this solution under stirring for about 10 min. The pH of
180 the chitosan solution was about 4.9. In parallel, 60g of gelatin was dissolved in 1L of
181 deionised water at $70^{\circ}C$ for 30 min under gentle stirring. 6.66 g of glycerol (10% w/w dry
182 matter) was added to the gelatin film forming. Subsequently, chitosan and gelatin film-
183 forming solutions were mixed at 1:1 ratio (w/w) at $50^{\circ}C$ (temperature of mixing) and stirred
184 for 30 min. The pH was adjusted to 5.5 on account of acetic acid. This condition was
185 specifically chosen to obtain a polyelectrolyte complex between chitosan and gelatin. At this
186 particular pH, the gelatin is negatively charged while the chitosan is positively charged,
187 favouring ionic interactions and avoiding any phase separation upon mixing. This complex

188 can only occur above the iso-electric point of the gelatin (Ip: 4.5-5.2, negatively charged) and
189 below the pKa of chitosan amino group is 6.2-6.5. When the film forming solution (FFS) was
190 completely homogenized, an aliquot of 30mL was then poured into plastic Petri dishes
191 (13.5cm in diameter). The aqueous solvent of the film forming solution was removed by
192 drying in a ventilated climatic chamber (KBF 240 Binder, ODIL, France) at 25°C and 45%
193 RH for 18 to 24 h. After drying, films were peeled off from the surface and stored up to
194 equilibration above silica gel or saturated saline solutions to control the relative humidity at
195 $\leq 2\%$, 53% and 84% before measuring aroma sorption.

196 **3.2. Sorption Kinetics of the aroma compounds**

197 In order to study the sorption kinetics of the aroma compounds by the matrices (films), the
198 appropriate conditions have been set up accordingly. The sorption is the result of ad- and ab-
199 sorption of the aroma vapours at the matrix surface from the vapour phase and its diffusion
200 through the matrix up to reach equilibrium. 20 pieces of 100 mg of each film were hung
201 across a metal grid attached to a wire and fixed at the top of the jars containing either silica
202 gel, or saturated salt solutions until equilibrium (4 days). Sheets of aluminium foil were
203 inserted in between each pieces of films to prevent sticking possibly due to moisture
204 absorption. The aroma compounds were then added at the bottom of the jars and tightly
205 closed. At this stage the sorption process started. Periodically, a piece of film was taken from
206 the jar, weighed and inserted into a glass vial containing 3g of acetone (4mL) used as the
207 extraction solvent. These vials were kept during 24h and at 25°C, followed by stirring till the
208 entire aroma compound was extracted. The extraction yield was previously determined and is
209 higher than 96%. The aroma compound extraction yield was taken into account for the final
210 calculation.

211 **3.3. Gas chromatography (GC) analysis of aroma compounds**

212 The quantity of aroma compounds sorbed by the film (expressed as mg of aroma /g of dry
213 matter of film) was carried out using a gas chromatograph (GC 2014, Shimadzu GmbH,
214 France). The chromatograph was equipped with a flame ionization detector (GC-FID).
215 Separation was performed on Agilent (J&W) DB-WAX capillary column (30 m length x 0.53
216 mm internal diameter x 1 μ m film thickness). The oven temperature (column) program was
217 set at gradient temperature: start by 50°C: equilibrium 1 min at 50°C, rate at 15°C/min to
218 reach 210°C isotherm during 5 min at 210°C. The injector and detector temperatures were at
219 240°C isotherm. Nitrogen was used as carrier gas (26 kPa) at 3mL/min, while hydrogen (25
220 mL/min) and air (250 mL/min) were used as ignition gases. The operating conditions were as
221 follows: split ratio 5; velocity 40 cm/sec; pressure 26 kPa.

222 For the aroma quantification in extraction solvent, 1 μ L from each vial was injected using a
223 gastight micro-syringe (Hamilton, Switzerland) and the analysis was carried out. The amount
224 of aroma extracted from the films was determined, in triplicate, using an external calibration
225 curve. This calibration curve (peak area as a function of concentration) was done, using the
226 same conditions, with pure aroma compounds dissolved in acetone at concentrations of 10,
227 50, 250, 500 and 1000 ppm. The concentration of the aroma compound in acetone is referred
228 to the dry weight of the film piece introduced in the acetone jar and expressed as mg of aroma
229 /g of dry matter of film.

230 **3.4. Thermal properties**

231 The Differential Scanning Calorimetry of films was studied using a DSC (Q20, TA
232 Instruments). The DSC analysis of the films was conducted both prior they were exposed to
233 aroma and RH atmospheres (control films) and after one month exposure. A piece of film,
234 around 5 mg sample was taken initially and after 1 month storage in the jars (sorption
235 kinetic), placed in an aluminium pan (10 μ L) hermetically sealed and directly subjected to a
236 double heating-cooling cycle. An empty aluminium pan was used as reference. Nitrogen was

237 used as the purge gas at a flow rate of 25 mL/min. Glass transition temperature (T_g) for each
238 sample was then determined from the mid-point of the second heating cycle using TA
239 Universal Analysis 2000 software (version 4.5 A, TA instruments). First heating above the
240 glass transition temperature and cooling at a controlled and adequate rate will erase bias due
241 to the mechanical stress induced by temperature variations (relaxation peaks). Therefore, the
242 second heating scan allowed to determine only the inherent thermal properties of our samples
243 such as the glass transitions and phase changes. The following temperature program then was
244 used for all samples:

245 - equilibration at 25 °C;

246 - cooling from 25°C to -80°C at the rate of 10°C/min followed by isothermal condition for 10
247 min;

248 - first heating to 150°C at a rate of 10°C/min;

249 - second cooling down to -80°C at a rate of 10°C/min followed by isothermal condition for
250 1min;

251 - second heating to 150°C at a rate of 10°C/min; and finally cooling down (drop) to 25°C.

252 Thermogravimetric analysis (TGA) was used to evaluate the thermal stability of the samples.

253 Measurements were conducted on films prior they were exposed to aroma and RH

254 atmospheres (control films), and after one month exposure, using a TA instrument (TA

255 instruments Discovery TGA New-Castle, USA). The temperature range was from 25 to

256 600°C, at a heating rate of 20°C/min, under nitrogen atmosphere. The weight of the film

257 sample was measured according the temperature with an accuracy of 0.01 mg. The

258 degradation temperature was determined from the derivative of the weight variation

259 according time (T_d: temperature of maximum transformation rate), for each film.

260 3.5. Statistical analyses

261 The data were analysed using an independent sample t-test with the statistical software SPSS
262 13.0 (SPSS Inc., Chicago, IL). A standard deviation (p-value < 0.05) at the 95% confidence
263 level was used to compare all the parameters analysed related to the Tg from DSC, the Td
264 from TGA and the amount of aroma compound sorbed after one month exposure to aroma
265 vapour according the relative humidity.

266

267 **4. Results and discussion**

268 **4.1. Sorption behaviours according aroma compounds properties and relative humidity**

269 The kinetics of aroma compound sorption by the chitosan-fish gelatin films at the three
270 relative humidity conditions ($\leq 2\%$, 53% and 84%) are given in Figure 1. Firstly, the sorbed
271 amount of aroma compounds (mg/g of dry matter) increased with times for all aroma
272 compounds studied except for 1-hexanal which behaviour is different. Except 2-hexen-1-ol
273 for which the sorption at RH $\leq 2\%$ RH occurred in two steps and 1-hexanal that presents a
274 maximum amount sorbed before a decrease, all the other compounds have an “ideal” sorption
275 behaviour (logarithmic increase of sorbed concentration) at the three relative humidities,. The
276 time to reach a plateau increased with increasing relative humidity value for 1-hexanol and 3-
277 hexanone: 7, 12 and 19 days for 1-hexanol and 6, 11 and 20 days for 3-hexanone,
278 respectively for RH $\leq 2\%$, and for 53% and 84% RH. A reverse tendency was observed for
279 phenol, in which the time to reach the plateau dropped with the increasing humidity. As
280 previously mentioned, 1-hexanal behaved differently. Indeed, 1-hexanal kinetic did not
281 displayed a clear plateau, but a maximum of sorption and then a decrease with time. The
282 maximum was about 5-7 days for RH $\leq 2\%$, and less than 1 day for higher relative humidities.
283 This peculiar behavior could be related to two or three phenomena that occurred at different
284 rates: 1°) sorption of 1-hexanal favoured or not by the presence of water (faster to reach
285 maximum sorption at higher RH), 2°) an antiplasticization of the polymer network by the 1-

286 hexanal which induced a decrease of 1-hexanal sorbed amount preponderantly at $RH \leq 2\%$.
287 Finally a competition between water and 1-hexanal sorption favoured a faster decrease of 1-
288 hexanal sorption at the higher relative humidities. Another hypothesis could involve carbonyl
289 function with the amino-acid residues of gelatin (well-known reaction between aldehyde and
290 proteins). This induces covalent bonds, which does not permit the further release of the 1-
291 hexanal that appeared, as a decrease with time because 1-hexanal linked to protein cannot be
292 extracted for the analysis.

293 1-Hexanal affected the structure properties of the network as confirmed latter by the thermal
294 analysis.

295 Whatever the chemical group, the amount of flavor sorbed by the film after one month of
296 exposure to a saturated atmosphere with different aroma compounds always increased. The
297 sorbed quantity varied from $0,31 \pm 0,07$ to $144,40 \pm 8,57$ mg/g dry film at $RH \leq 2\%$, from
298 $0,43 \pm 0,02$ to $511,53 \pm 32,25$ mg/g dry film at 53% RH and from $1,04 \pm 0,12$ to $519,80 \pm 40,74$
299 mg/g dry film at 84% RH (Figure 2). The sorbed amount was significantly ($p < 0,05$) increased
300 with RH value after one month exposure for the 3-hexanone, phenol and 1-hexanal. The
301 increase in moisture enhanced the sorption from 3 to 6 times respectively for these three
302 aroma compounds. On the contrary, for the two aliphatic alcohols, the sorbed amount slightly
303 decrease when the RH rose from $RH \leq 2\%$ to 53% RH (Figure 2). This decrease should be
304 related to the competition with water to occupy the free binding sites on chitosan and gelatin
305 chains. Zhou & Cadwallader (2006) demonstrated that for the relatively polar volatile aroma
306 (2-hexanone, trans-2-hexenal, 1-hexanol, trans-2-hexen-1-ol), their interaction with soy
307 protein decreased when the RH level increased from 0 to 30%, suggesting that competition
308 for high-energy binding sites between flavor compound and water exists. Water can readily
309 and tightly bind with polar or hydrophilic biopolymers, and once water occupies high-energy
310 polar binding sites, it is thus not readily displaced. However, at the higher RH (84%), sorbed

311 amount dramatically increased from 7 to 28 times respectively for 2-hexen-1-ol and 1-
312 hexanol. Sorbed amount of phenol also increased with moisture (Figure 2). This can be
313 explained by the polar character of alcohols which occupy preferently the hydrophilic binding
314 sites of the biopolymers.

315 The presence of water vapour often accelerates the diffusion of volatiles aroma in water
316 sensitive polymers such as gelatin and chitosan. The water diffuses into the film and acts like
317 a plasticizer, opening the polymer structure to molecular transport of the aroma compounds.
318 Furthermore, it was suggested that the increase in aroma sorption with RH value was due to
319 the moisture plasticization phenomenon for most of the hydrophilic edible films (Debeaufort
320 & Voilley, 1994). Brody (2002) studied the scalping of aroma compounds in plastic
321 packaging. They showed that an increase in moisture content of moisture-sensitive plastics
322 such as polyamides or EVOH significantly increased the permeation rate of aroma
323 compounds.

324 In addition, the Tg values for the control film decreased from 9.53°C to -13.37°C when RH
325 value risen from RH \leq 2 to 84% RH (Table 2 and Figure 3) as usually observed for the
326 hydrophilic bio-polymers. Film matrix became more rubbery (Tg below ambient
327 temperature), and therefore the polymer molecules were highly flexible, favoring both
328 diffusion and sorption of small molecules like solutes or aroma compounds.

329 Whatever the relative humidity condition, the order of sorption is keton (3-hexanone)
330 <aldehyde (1-hexanal) <aliphatic alcohols (2-hexen-1-ol and 1-hexanol) < phenol. When we
331 compare the 6 carbon chain compounds the results were significantly different ($p < 0.05$), even
332 if their chains had the same carbon number. This behavior could be mainly related to the
333 vapour pressure and polarity (solubility in water). Indeed, the lower is the vapour pressure,
334 and the higher is the solubility, the higher is the sorption. Phenol with low vapor pressure (0.4
335 mmHg) presents a great affinity towards polymer chains and is the most sorbed by films. On

336 the contrary, the aromas with the highest vapor pressure (3-hexanone: 11 mmHg and 1-
337 hexanal: 10 mmHg) were weakly sorbed. This finding was also confirmed by (Fabra,
338 Hambleton, Talens, Debeaufort, Chiralt, & Voilley, 2008) who displayed that the low
339 saturated vapour pressure make easier the sorption of aroma compounds by edible films.

340 Boiling point and flash point are characteristics related to the vapour pressure and molecular
341 weight. Boiling point is indicative of the ability of a volatile molecule to condense and
342 remain within the matrix. Phenol with the higher boiling point (140°C), is the most sorbed;
343 whereas 3-hexanone and 1-hexanal with the lowest boiling points (126 and 127°C) are the
344 less sorbed. The higher the boiling point, the higher sorption of aroma in polymer (Sajilata,
345 Savitha, Singhal, & Kanetkar, 2007). Fayoux, Seuvre, & Voilley (1997) also noticed that the
346 effect of the molecular weight or the boiling point on aroma transport in and through plastic
347 packaging was more important than the functional group nature.

348 The same trend was observed for the experimental flash point: 3-hexanone and 1-hexanal
349 with the lowest flash point (23 and 25°C) are sorbed at fewer amounts; nonetheless, phenol
350 with the highest flash point (79°C) is greatly sorbed.

351 Besides, 3-hexanone and 1-hexanal with the higher molar volume (124 and 122.5 mL/mole)
352 were sorbed in the smallest quantities by film matrix compared to phenol with the lowest
353 molar volume (89 mL/mole), this latter was sorbed to a greater extent. Here again, the
354 possible covalent binding between the aldehyde and the gelatin did not permit its extraction
355 and thus only a weak part (non covalently bind) of the sorbed 1-hexanal was released.

356 Polarity and affinity are often related to chemical structure, solubility, logP, surface tension,
357 and also Kovats and retention indexes. Linssen, Verheul, Roozen, & Posthumus (1992)
358 reported that highly branched and cyclic molecules were absorbed to a greater extent than
359 linear molecules by plastic films. The sorbed amount of aliphatic alcohols is between 4 and
360 60 times higher than that observed for keton and aldehyde, regardless the relative humidity

361 conditions. This stronger interaction forces between aroma and polymer chains, observed for
362 the two alcohols suggest that high-energy hydrogen bonding and/or more than one hydrogen
363 bond was involved. This is possible because of the H donor and acceptor nature of the
364 hydroxyl group compared to keton and aldehyde group (Zhou & Cadwallader, 2006). In
365 addition, the two aliphatic alcohols (2-hexen-1-ol and 1-hexanol) were more flexible, less
366 bulky molecule than keton (3-hexanone), which would facilitate its ability to move into the
367 rubbery polymer matrix. Moreover, the sorption of 2-hexen-1-ol was higher than 1-hexanol,
368 indicating that the presence of a double bond together with a strongly interacting functional
369 group (such as hydroxyl group) could have a significant influence on flavor-polymers
370 interaction. Zhou & Cadwallader (2006), studied the effect of the chemical structure of some
371 flavor compounds on their binding to soy protein isolate. They displayed that the restricted
372 rotation of the double bond on 2-hexen-1-ol induces the molecule rigidity increase, which
373 enhances the exposure of the hydroxyl group. Hence, its interaction was potentially increased
374 with soy proteins compared to 1-hexanol (without double bond).

375 Indeed, flavors are absorbed more easily in a polymeric film of similar polarity (Quezada
376 Gallo, Debeaufort, & Voilley, 1999). However, very few authors studied the effect of
377 polarities on the aroma and biopolymer transfer and the sorption in hydrophilic edible
378 packaging. Sajilata, Savitha, Singhal, & Kanetkar (2007) displayed that aldehyde and keton
379 are low and middle polar aroma molecules but alcohol is a more polar molecule. These
380 findings confirm our results: alcohols (2-hexen-1-ol and 1-hexanol) were more sorbed than
381 the aldehyde (1-hexanal) and keton (3-hexanone), considering the polar nature of hydrophilic
382 films. In the case of the aldehyde, only non-covalently bind molecule were analyzed. Among
383 alcohol, phenol behave differently due to its cyclic structure.

384

385 **4.2. Modification of film structure and properties due to sorbed aroma compounds**

386 The change in the structure properties of film induced by the sorption of aroma was assessed
387 from thermal analysis (DSC). Indeed, the second heating cycle thermograms of chitosan-fish
388 gelatin films after one month of exposure to pure aroma compounds vapour (1-hexanol, 2-
389 hexen-1-ol, 3-hexanone, phenol and 1-hexanal) at the three relative humidities are given in
390 **Figure 3**.

391 The glass transition temperature (T_g) and the degradation temperature (T_d) for each films
392 was displayed in **Table 2**. Firstly we can observe the plasticization effect of the biopolymer
393 matrix by the water sorption. Indeed, water induced a significant decrease of the T_g value of
394 the control films from $+9.53^\circ\text{C}$ to -13.37°C when the RH increased from $\text{RH} \leq 2$ to 84% RH.
395 This finding is also observed by [Giacin & Hernandez \(1997\)](#) who displayed that the
396 semicrystalline polyolefins (PE and PP) are non sensitive to moisture. Indeed, semi
397 crystalline PE and PP have T_g below the ambient temperature, have higher diffusion
398 coefficient for flavors and higher gaz permeation revealing polymer network plasticization by
399 the flavours. Phenol presence displayed a decrease of the degradation temperature compared
400 to the control films whatever the RH value. Indeed, phenol induced a plasticization which
401 favoured the chain mobility and thus the temperature sensitivity of the film network. The
402 water seems not to affect the film stability when phenol is present. The films are already
403 plasticized by the phenol prior sorption of the water (RH increasing). For the other
404 compounds, the moisture seems to increase (but not significantly) or did not affect the T_d ,
405 except for 2-hexen-1-ol. The antiplasticization of the network by the aroma compounds
406 dominated the effect of the plasticization by the moisture. In the case of 1-hexanal, the films
407 were crosslinked by the aroma compound. In that case, the effect of the water on the T_d only
408 occurred at the higher RH values.

409 However, when the films were exposed to both moisture and aroma vapours, different
410 behaviours have been observed according the RH and nature of aroma compounds.

411 Phenol is highly soluble in the films and consequently it is an efficient plasticizer as it
412 reduced the T_g by almost 55°C at RH ≤2%, 26°C at 53% RH and 40°C at 84% RH compared
413 to the control film. The less plasticizing effect of phenol at highest RH was due to the
414 preferential sorption of water by the matrix. At the same time, the degradation temperature
415 decrease by more than 7°C whatever the relative humidity condition when films was exposed
416 to phenol. This reveals the predominant plasticizing effect of phenol.

417 Whatever the RH, 1-hexanol, 2-hexen-1-ol, 3-hexanone and 1-hexanal have an
418 antiplasticizing effect revealed by the increase of the films T_g compared to the control films,
419 even for very low amount sorbed. However, 2-hexen-1-ol at 53% RH seemed to have a very
420 low antiplasticizing or no effect (T_g decrease by only 2°C).

421 The antiplasticization of films by the aroma compounds have been confirmed by more than
422 10°C increase of the T_d at RH ≤2% and 53% RH for 1-hexanal, and at 53% and 84% RH for
423 2-hexen-1-ol (Table 2). However, there is no significant effect of 3-hexanone and 1-hexanol
424 on the T_d values compared to control films. For these two compounds, the amounts of aroma
425 sorbed by the films are probably too low to affect their thermal stability measured by TGA.

426 The alcohol residues (1-hexanol, 2-hexen-1-ol) may interact with the lateral chains of
427 chitosan and gelatin and could form hydrogen bonds inducing a film stiffness rise. The
428 ketone group (3-hexanone) interacts with the lateral OH group of polysaccharides as
429 (Quezada-Gallo, Debeaufort, & Voilley, 2000) had shown in the case of iota-carrageenan
430 films exposed to 2-hexanone.

431 In addition, at higher RH and with the sorbed amount increase, the antiplasticizing effect
432 became more important (great increase of T_g about 7, 9 and 15°C, respectively for 1-hexanol,
433 2-hexen-1-ol and 3-hexanone).

434 For 1-hexanol, from RH ≤2% to 53% RH, the sorption of the aroma induced an
435 antiplasticization as the T_g rose from 16.4 to 27.8°C but at 84% RH, the sorption of water

436 was predominant and thus its plasticization effect dominated the aroma antiplasticization. For
437 the 3-hexanone, when RH increased from RH ≤ 2 to 53% RH, almost no effect of the moisture
438 on the Tg was observed. This is probably due to the very low amount sorbed for this
439 compound. However, at 84% RH, though the water vapour sorption occurred, the greater
440 sorption of 3-hexanone still induced an antiplasticization of the film by this aroma compound.
441 The Tg of film exposed to 1-hexanal at 84% RH cannot be determined. But a great increase
442 of Tg by 70°C was observed compared to control films at 53% RH. We can suspect the trend
443 was maintained for higher RH even if not observed from the thermogram. Indeed, films
444 exposed to 1-hexanal revealed an increase of Tg by about 65°C when the RH rose from RH
445 ≤ 2 to 53% RH, the sorption of the aroma induced an antiplasticization effect.

446

447 **Conclusion**

448 Results from this study demonstrated that the chemical structure of volatile flavor compounds
449 greatly determined its binding with hydrophilic films and that relative humidity had a
450 substantial influence on the interaction potential of apolar/polar flavor compounds.

451 According the physical-chemical characteristics of the aroma compounds, the kinetics of
452 sorption varied with the level of moisture and some peculiar behaviours were observed,
453 revealing either competition or synergy between the water and aroma sorption, and revealing
454 either plasticization or antiplasticization effect.

455 The quantity of sorbed of aroma compound is not directly linked to the structural changes of
456 the biopolymer network observed (plasticization or antiplasticization). Whatever the level of
457 RH, aliphatic alcohols, keton and aldehyde acts as antiplasticizer despite of the low amount
458 sorbed at equilibrium. On the contrary, phenol always plasticized the fish-gelatin chitosan
459 film network, but in a competitive behavior with water.

460 A peculiar behaviour was observed for 1-hexanal in which the kinetic **occurred** at two steps, a
461 very fast sorption and high amount sorbed and then an apparent decrease of sorbed quantity
462 with time. In fact, there was no extraction of this compounds because the sorbed 1-hexanal
463 interacted with amine group of gelatin to form covalent bonds that did not permit its release
464 from the polymers chains.
465 Finally, chitosan-gelatin films looks very interesting for their application as barrier to
466 aliphatic aroma compounds for packaging films.

467

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544 environmental relative humidity on the binding of volatile flavor compounds to
545 dehydrated soy protein isolates. *J Agric Food Chem*, 54(5), 1838-1843.

546

547

548

549 **Figure captions**

550 Figure 1: Kinetic of aroma compound sorption by the chitosan-fish gelatin films at three
551 relative humidity conditions ($\leq 2\%$, 53% and 84%) and at 25°C (hand-plot lines are only eye-
552 guides).

553 Figure 2: Aroma compound sorbed (mg /g of dry matter) and at 25°C by chitosan-fish gelatin
554 films after one month exposure to saturated vapour of aroma compound as a function of the
555 relative humidity ($\leq 2\%$, 53% and 84%).

556 Figure 3: Thermograms (second heating cycle) for chitosan-fish gelatin films after one
557 month of exposure at 25°C in saturated atmospheres of the pure aroma compounds (1-
558 hexanol, 2-hexen-1-ol, 3-hexanone, phenol and 1-hexanal) at the three relative humidities
559 ($\leq 2\%$, 53% and 84%, the arrows are eye-guides of the Tg mid point)

560

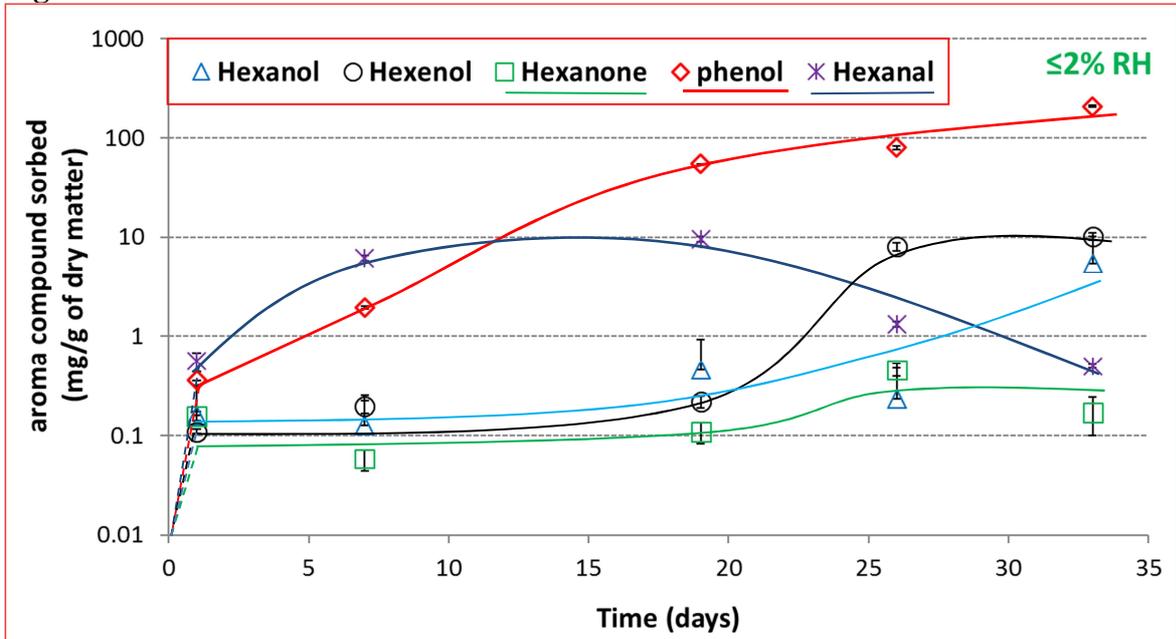
561 **Table captions**

562 Table 1: Structural and physicochemical properties of the aroma compounds (1-hexanol, 2-
563 hexen-1-ol, 3-hexanone, phenol and 1-hexanal, data from www.chemspider.com).

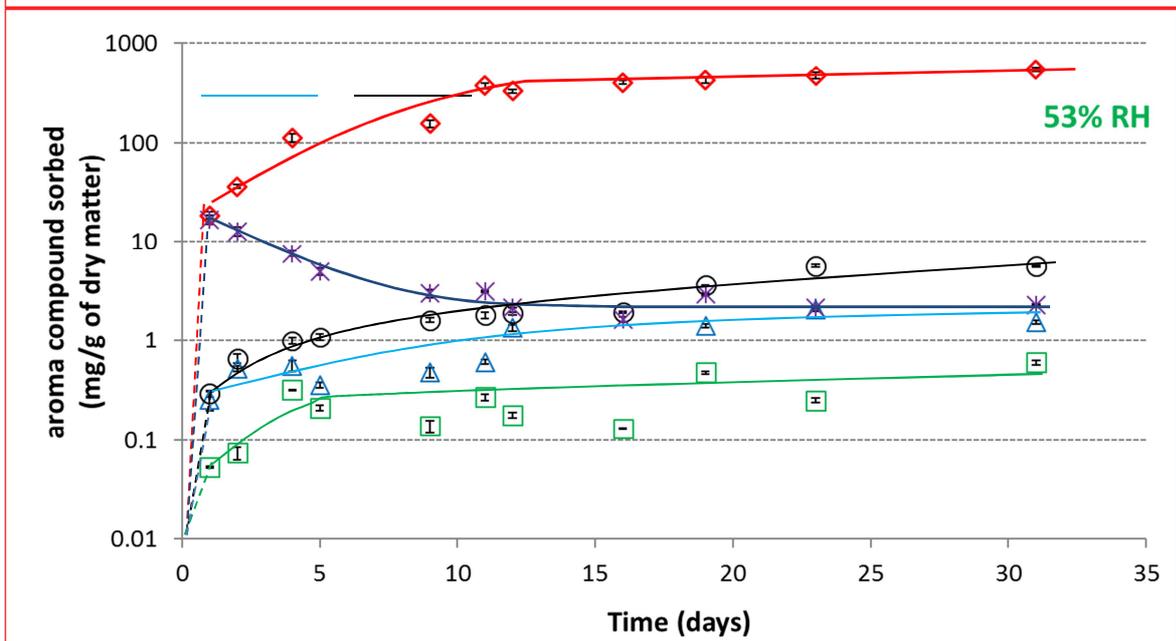
564 Table 2: Glass transition temperature (Tg) determined from the DSC second heating **and the**
565 **degradation temperature (Td) determined from the TGA thermogram for each chitosan-fish**
566 **gelatin films before and** after one month of exposition to an aroma saturated atmosphere (1-
567 hexanol, 2-hexen-1-ol, 3-hexanone, phenol and 1-hexanal) at the three relative humidity (RH)
568 conditions ($\leq 2\%$, 53% and 84%).

569

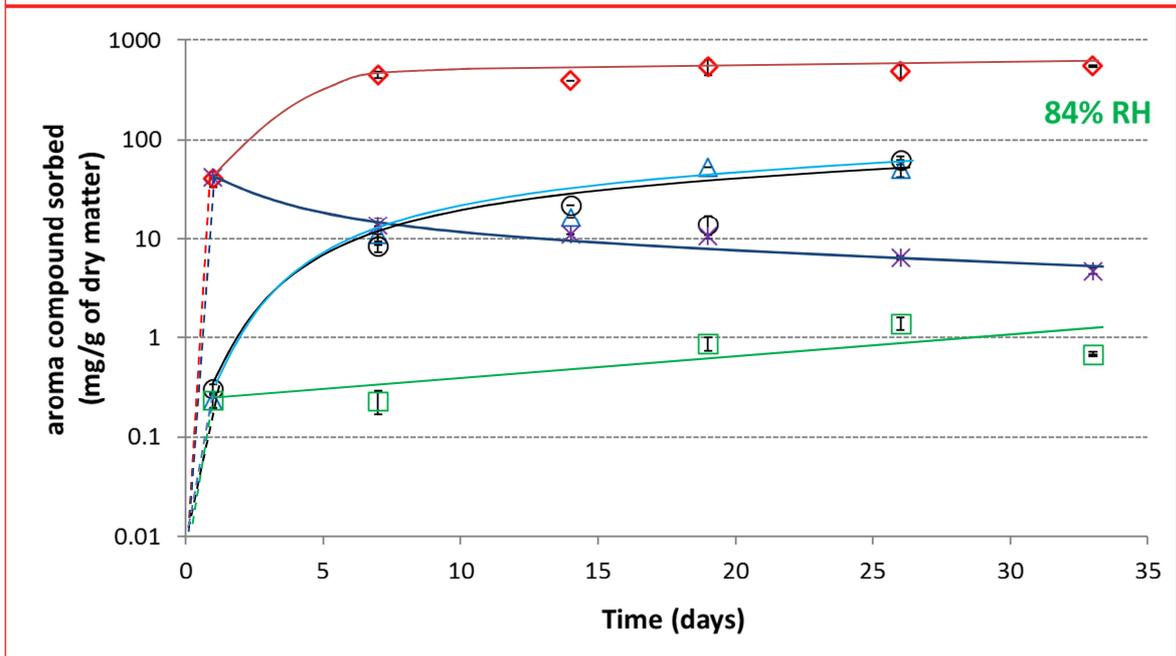
1 **Figure 1**



2

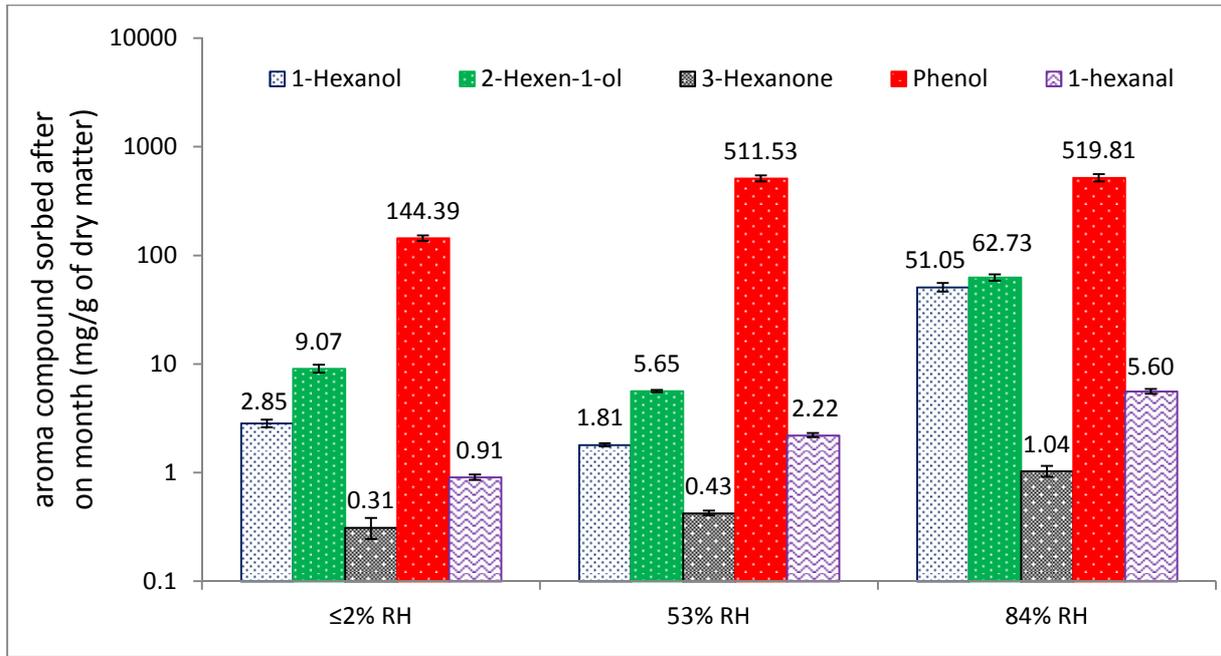


3

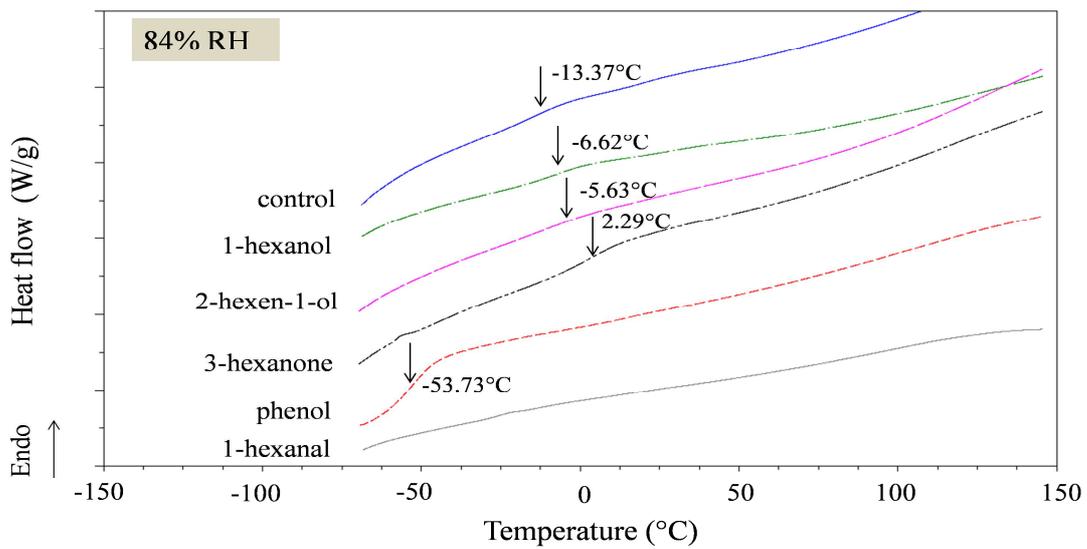
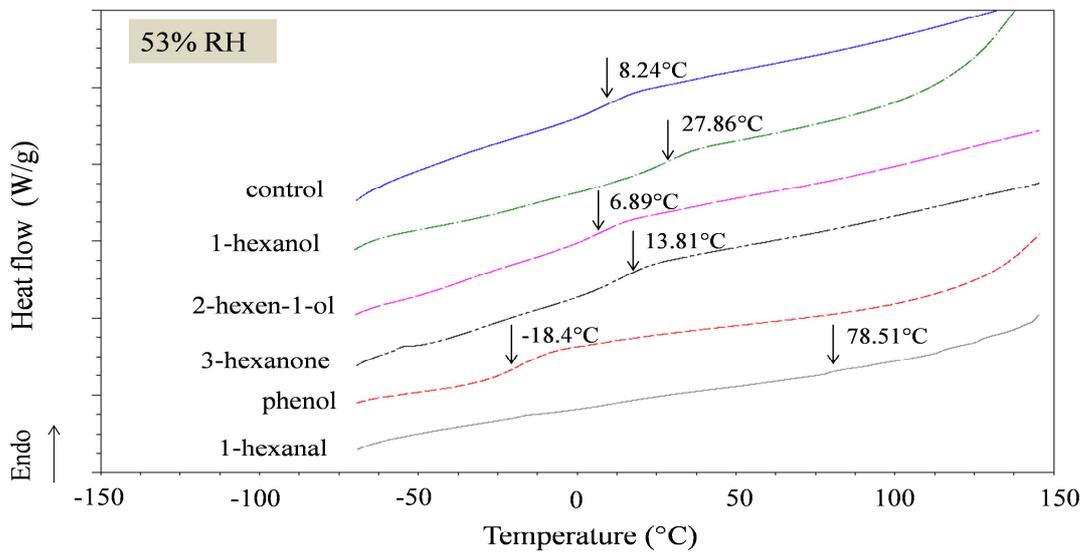
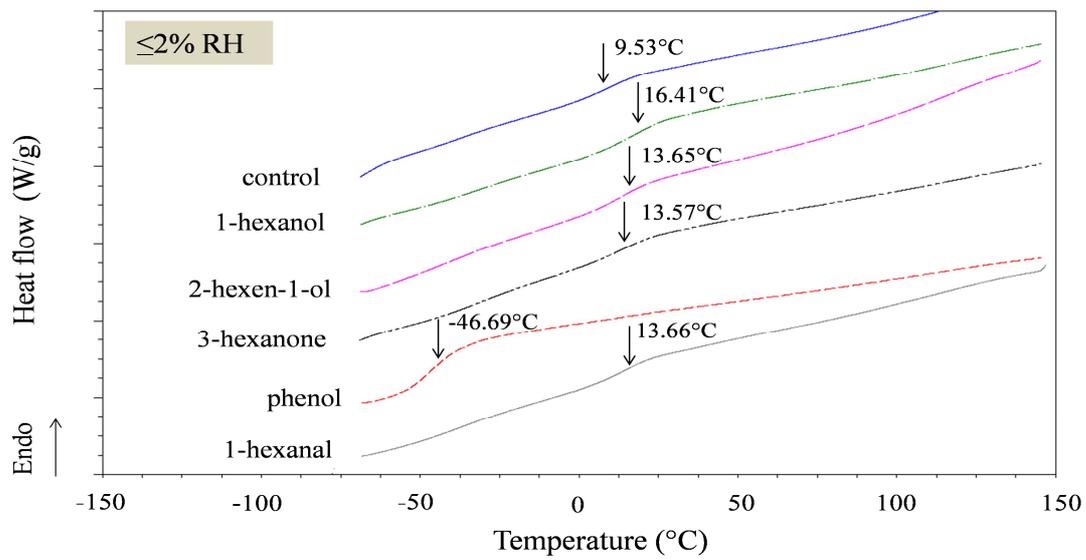


4

1 **Figure 2**



1 Figure 3



- 1 **Table 1:** Structural and physicochemical properties of the aroma compounds (1-hexanol, 2-hexen-1-ol, 3-hexanone, phenol and 1-hexanal, data
- 2 from www.chemspider.com).

Chemical name and structure	1-Hexanol	2-Hexen-1-ol	3-Hexanone	Phenol	1-hexanal
-----------------------------	------------------	---------------------	-------------------	---------------	------------------

Molecular formula	C ₆ H ₁₄ O	C ₆ H ₁₂ O	C ₆ H ₁₂ O	C ₆ H ₆ O	C ₆ H ₁₂ O
Average mass (Da)	102.175	100.159	100.159	94.111	100.16
Molar volume (mL/mol)	125	118.7	124.1	89	122.5
Experimental melting point (°C)	-52	54	-56	43	-58.2
Experimental boiling point (°C)	157	140.4	126	181	127
Experimental vapor pressure (mmHg)	1	2.5	11	0.4	10
Experimental LogP	2.03	2.38	1.43	1.46	1.78
Experimental flash point (°C)	60	47.7	23	79	25
Solubility in water (mg/L)	5900	1800	7700	20000	3500
Evaporation temperature (°C)*	116	110	69	114	65

4 (*) Evaporation temperature (°C) determined from TGA analysis of pure aroma compounds tested

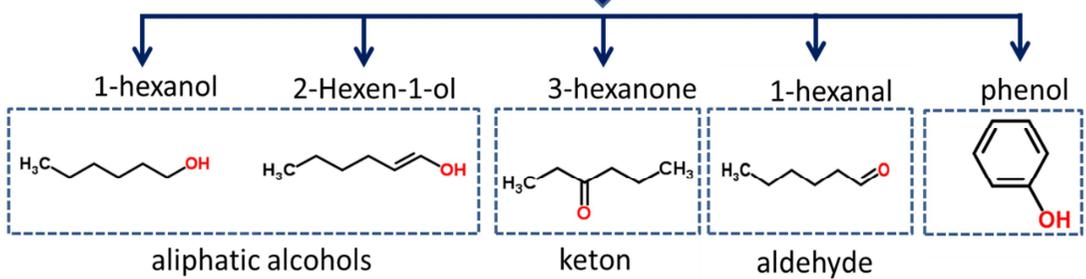
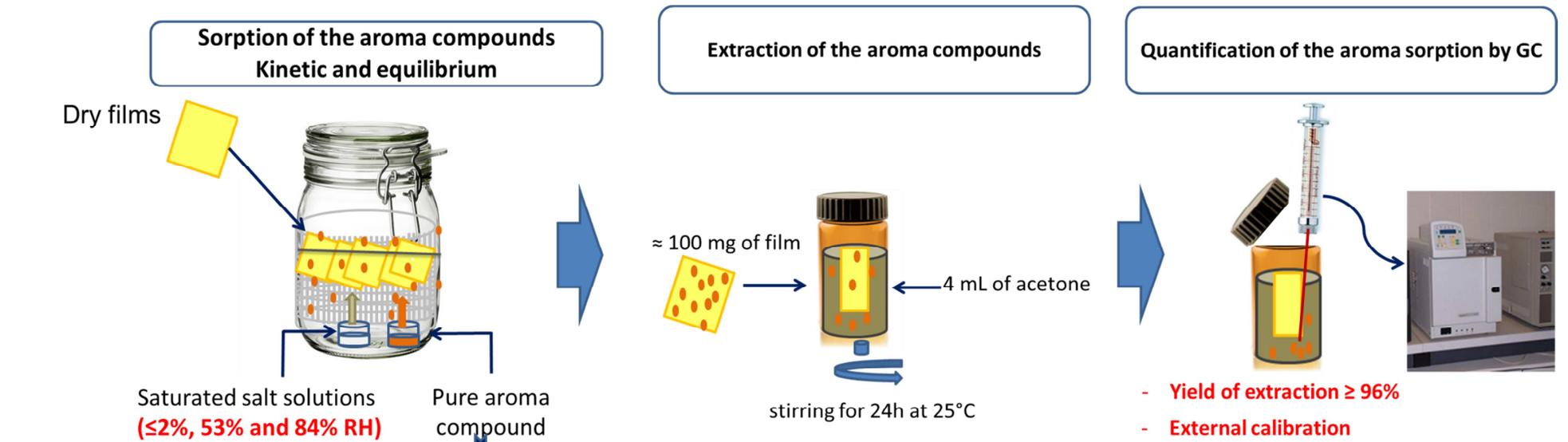
1 **Table 2:** Glass transition temperature (T_g) determined from the DSC second heating and the degradation temperature (T_d) determined from the
 2 TGA thermogram for each chitosan-fish gelatin films before and after one month of exposition to an aroma saturated atmosphere (1-hexanol, 2-
 3 hexen-1-ol, 3-hexanone, phenol and 1-hexanal) at the three relative humidity (RH) conditions ($\leq 2\%$, 53% and 84%).

Films		$\leq 2\% \text{ RH}$	53 % RH	84% RH
Control film	T _g	9.53 ^c	8.24 ^c	-13.37 ^c
	T _d	278 ^b	275 ^b	274 ^b
Film exposed to 1-hexanol	T _g	16.41 ^f	27.86 ^g	-6.62 ^c
	T _d	271 ^{a,b}	273 ^{a,b}	278 ^b
Film exposed to 2-hexen-1-ol	T _g	13.65 ^{e,f}	6.89 ^e	-5.63 ^c
	T _d	274 ^b	284 ^{b,c}	301 ^c
Film exposed to 3-hexanone	T _g	13.57 ^{e,f}	13.81 ^{e,f}	2.29 ^d
	T _d	274 ^b	274 ^b	277 ^b
Film exposed to phenol	T _g	-46.69 ^{a,b}	-18.40 ^c	-53.73 ^a
	T _d	267 ^a	268 ^a	267 ^a
Film exposed to 1-hexanal	T _g	13.66 ^{e,f}	78.51 ^h	N.D.
	T _d	289 ^c	289 ^c	276 ^b

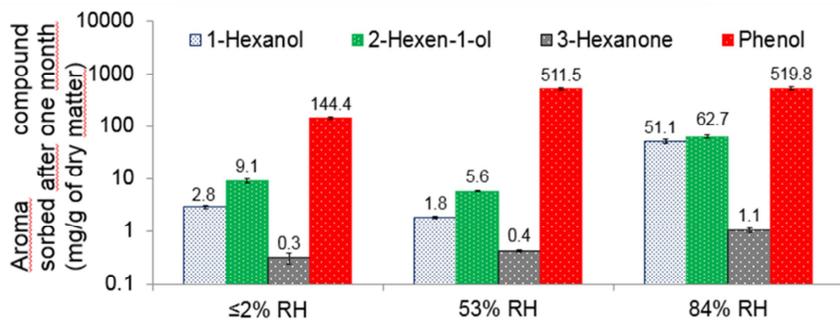
4 N.D. not detectable

5 a,b,c,d,e,f,g,h: values having the same letters for each parameter (T_g and T_d) are not significantly different at p level =0.05

6



Quantity sorbed is moisture dependent



Film structure affected by sorbed aroma compounds

