Sorption kinetic of aroma compounds by edible bio-based films from marine-by product macromolecules: Effect of relative humidity conditions

Nasreddine Benbettaieb, Claire O'Connell, Anne-Sophie Viaux, Elias Bou-Maroun, Anne-Marie Seuvre, Claire-Hélène Brachais, Frédéric Debeaufort

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

Nasreddine Benbettaïeb[1&2], Claire O'Connell [3], Anne-sophie Viaux[1], Elias Bou-Maroun[1]
Anne-Marie Seuvre[1&2] Claire-Hélène Brachais [4], Frédéric Debeaufort[1&2 *]

[1] IUT-Dijon-Auxerre, Dpt BioEngineering, 7 blvd Docteur Petitjean, 20178 Dijon Cedex, France

[2] Univ. Bourgogne Franche-Comté, AgroSup Dijon, PAM UMR A 02.102, F-21000 Dijon, France

[3] Cork Institute of Technology, Dpt of BioSciences, Rossa Av., Bishopstown, Cork, Ireland

[4] Univ. Bourgogne Franche-Comté, Institut de chimie moléculaire de l'université de Bourgogne ICMUB- UMR6302 uB – CNRS, 9 avenue Alain Savary, 21078 Dijon Cedex, France

* Corresponding author:
Univ. Bourgogne Franche-Comté, AgroSup Dijon, PAM UMR A 02.102, F-21000 Dijon, France
Tel: +33 380 77 2388; fax: +33 380 77 4011.
Author's e-mail: frederic.debeaufort@u-bourgogne.fr (F. Debeaufort)

Abstract

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

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

1. Introduction

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

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

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

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

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

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

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

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

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

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

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

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

2. Materials and reagents

A commercial food grade chitosan (France Chitine) and a commercial grade fish gelatin (Rousselot 200 FG), were the filmogenic biopolymer used for the film matrix. The chitosan (CS) characteristics are: MW=165 kDa, low viscosity, 85% deacetylation degree), and those of fish gelatine (FG) are: 180 Bloom degree, a 4 mPas viscosity at 45^0C and for a concentration of 6.67% in water and at pH=5.4.

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

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

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

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

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

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

- dried silicagel (WVR France, 98% purity) for RH ≤ 2% at 25°C,
- NaBr (Sigma-Aldrich, 99% purity) saturated salt solution for 53% RH at 25°C
- And KCl (Sigma-Aldrich, 99% purity) saturated salt solution for 84% RH at 25°C

Silicagel was dried at 105°C for one night and saturated solutions freshly prepared prior using.

3. Methods

3.1. Film Preparation

A chitosan solution was prepared by dispersing 20g of the chitosan powder in 1L of the 1% acetic acid aqueous solution. The solution was homogenised at 1200rpm with a high shear homogeniser Ultra Turrax (RW16 basic IKA – WERKE). 2.22g of glycerol (10% w/w dry matter polymer) was then added to this solution under stirring for about 10 min. The pH of the chitosan solution was about 4.9. In parallel, 60g of gelatin was dissolved in 1L of deionised water at 70°C for 30 min under gentle stirring. 6.66 g of glycerol (10% w/w dry matter) was added to the gelatin film forming. Subsequently, chitosan and gelatin film-forming solutions were mixed at 1:1 ratio (w/w) at 50°C (temperature of mixing) and stirred for 30 min. The pH was adjusted to 5.5 on account of acetic acid. This condition was specifically chosen to obtain a polyelectrolyte complex between chitosan and gelatin. At this particular pH, the gelatin is negatively charged while the chitosan is positively charged, 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 ≤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**

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

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

3.4. Thermal properties

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

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

- equilibration at 25 °C;
- cooling from 25°C to -80°C at the rate of 10°C/min followed by isothermal condition for 10 min;
- first heating to 150°C at a rate of 10°C/min;
- second cooling down to -80°C at a rate of 10°C/min followed by isothermal condition for 1min;
- second heating to 150°C at a rate of 10°C/min; and finally cooling down (drop) to 25°C.

Thermogravimetric analysis (TGA) was used to evaluate the thermal stability of the samples. Measurements were conducted on films prior they were exposed to aroma and RH atmospheres (control films), and after one month exposure, using a TA instrument (TA instruments Discovery TGA New-Castle, USA). The temperature range was from 25 to 600°C, at a heating rate of 20°C/min, under nitrogen atmosphere. The weight of the film sample was measured according the temperature with an accuracy of 0.01 mg. The degradation temperature was determined from the derivative of the weight variation according time (Td: temperature of maximum transformation rate), for each film.

3.5. Statistical analyses

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

4. Results and discussion

4.1. Sorption behaviours according aroma compounds properties and relative humidity

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

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

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

Whatever the chemical group, the amount of flavor sorbed by the film after one month of exposure to a saturated atmosphere with different aroma compounds always increased. The sorbed quantity varied from 0,31±0.07 to 144.40±8.57 mg/g dry film at RH ≤2%, from 0.43±0.02 to 511.53±32.25 mg/g dry film at 53% RH and from 1.04±0.12 to 519.80±40.74 mg/g dry film at 84% RH (Figure 2). The sorbed amount was significantly ($p<0.05$) increased with RH value after one month exposure for the 3-hexanone, phenol and 1-hexanal. The increase in moisture enhanced the sorption from 3 to 6 times respectively for these three aroma compounds. On the contrary, for the two aliphatic alcohols, the sorbed amount slightly decrease when the RH rose from RH ≤2% to 53% RH (Figure 2). This decrease should be related to the competition with water to occupy the free binding sites on chitosan and gelatin chains. Zhou & Cadwallader (2006) demonstrated that for the relatively polar volatile aroma (2-hexanone, trans-2-hexenal, 1-hexanol, trans-2-hexen-1-ol), their interaction with soy protein decreased when the RH level increased from 0 to 30%, suggesting that competition for high-energy binding sites between flavor compound and water exists. Water can readily and tightly bind with polar or hydrophilic biopolymers, and once water occupies high-energy 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 ≤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 convalently 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

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

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

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 (Tg) and the degradation temperature (Td) 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 Tg value of
394 the control films from +9.53°C to -13.37°C when the RH increased from RH ≤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 Tg 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 Td,
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 Td 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.

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

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

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

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

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

For 1-hexanol, from RH ≤2% to 53% RH, the sorption of the aroma induced an antiplasticization as the Tg 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.

A peculiar behaviour was observed for 1-hexanal in which the kinetic occurred at two steps, a very fast sorption and high amount sorbed and then an apparent decrease of sorbed quantity with time. In fact, there was no extraction of this compounds because the sorbed 1-hexanal interacted with amine group of gelatin to form covalent bonds that did not permit its release from the polymers chains.

Finally, chitosan-gelatin films looks very interesting for their application as barrier to aliphatic aroma compounds for packaging films.

Acknowledgements

The authors wish to thank the colleagues from the PAM-PAPC Laboratory for precious collaboration and help, and to thank ESIREM (Engineering School of Materials of the université de Bourgogne for the free access to equipment and devices. This work was supported by the Regional Council of Bourgogne Franche-Comté and the "Fonds Européen de Développement Régional (FEDER)".

References

Balaguer, M. P., Gavara, R., & Hernández-Muñoz, P. (2012). Food aroma mass transport properties in renewable hydrophilic polymers. *Food Chemistry, 130*(4), 814-820.

Benbettaïeb, N., Tanner, C., Cayot, P., Karbowiak, T., & Debeaufort, F. (2018). Impact of functional properties and release kinetics on antioxidant activity of biopolymer active films and coatings. *Food Chemistry, 242*(Supplement C), 369-377.

Bordenave, N., Grelier, S., Pichavant, F., & Coma, V. (2007). Water and moisture susceptibility of chitosan and paper-based materials: structure-property relationships. *J Agric Food Chem, 55*(23), 9479-9488.

485 Brody, A. L. (2002). Flavor scalping: Quality loss due to packaging. *Food Technology*, *56*(6),
486 124-125.
487 Caner, C. (2011). Sorption Phenomena in Packaged Foods: Factors Affecting Sorption
488 Processes in Package–Product Systems. *Packaging Technology and Science, 24*(5), 259-
489 270.
490 Debeaufort, F., & Voilley, A. (1994). Aroma compound and water vapor permeability of
491 edible films and polymeric packagings. *Journal of Agricultural and Food Chemistry,*
492 *42*(12), 2871-2875.
493 Dury-Brun, C., Chalier, P., Desobry, S., & Voilley, A. (2007). Multiple Mass Transfers of
494 Small Volatile Molecules Through Flexible Food Packaging. *Food Reviews*
495 *International*, *23*(3), 199-255.
496 Fabra, M., Hambleton, A., Talens, P., Debeaufort, F., Chiralt, A., & Voilley, A. (2008).
497 *Aroma Barrier Properties of Sodium Caseinate-Based Films* (Vol. 9).
498 Fayoux, S. C., Seuvre, A. M., & Voilley, A. J. (1997). Aroma transfers in and through plastic
499 packagings: orange juice and d-limonene. A review. Part II: overall sorption mechanisms
500 and parameters—a literature survey. *Packaging Technology and Science, 10*(3), 145-160.
501 Giacin, J. R., & Hernandez, R. J. (1997). Permeability of aromas and solvents in polymeric
502 packaging materials.In: Brody AL, Marsh KS, editors *The Wiley Encyclopedia of*
503 *packaging technology. New York: John Wiley & Sons Inc*, 724-733.
504 Hoque, M. S., Benjakul, S., & Prodpran, T. (2011). Properties of film from cuttlefish (*Sepia*
505 *pharaonis*) skin gelatin incorporated with cinnamon, clove and star anise extracts. *Food*
506 *Hydrocolloids*, *25*(5), 1085-1097.
507 Jridi, M., Sellimi, S., Lassoued, K. B., Beltaief, S., Souissi, N., Mora, L., Toldra, F., Elfeki,
508 A., Nasri, M., & Nasri, R. (2017). Wound healing activity of cuttlefish gelatin gels and

films enriched by henna (Lawsonia inermis) extract. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 512*, 71-79.

Kester, J. J., & Fennema, O. (1989). An Edible Film of Lipids and Cellulose Ethers: Performance in a Model Frozen-Food System. *Journal of Food Science, 54*(6), 1390-1392.

Leelaphiwat, P., Auras, R. A., Harte, J. B., Ong, P. K. C., & Chonhenchob, V. (2016). Barrier Properties of Polymeric Packaging Materials to Major Aroma Volatiles in Herbs. *MATEC Web Conf., 67*, 06100.

Linssen, J. P. H., Verheul, A., Roozen, J. P., & Posthumus, M. A. (1992). Absorption of flavour compounds by packaging material: Drink yoghurts in polyethylene bottles. *International Dairy Journal, 2*(1), 33-40.

Miller, K. S., & Krochta, J. M. (1997). Oxygen and aroma barrier properties of edible films: A review. *Trends in Food Science & Technology, 8*(7), 228-237.

Moradi, M., Tajik, H., Razavi Rohani, S. M., Oromiehie, A. R., Malekinejad, H., Aliakbarlu, J., & Hadian, M. (2012). Characterization of antioxidant chitosan film incorporated with Zataria multiflora Boiss essential oil and grape seed extract. *LWT - Food Science and Technology, 46*(2), 477-484.

Park, S.-i., & Zhao, Y. (2004). Incorporation of a High Concentration of Mineral or Vitamin into Chitosan-Based Films. *Journal of Agricultural and Food Chemistry, 52*(7), 1933-1939.

Quezada-Gallo, A., Debeaufort, F., & Voilley, A. (2000). In Food Packaging: Testing Methods and Applications (Risch, S.J. ed.). *ACS Symposium Series, 753*, 125-140.

Quezada Gallo, Debeaufort, & Voilley. (1999). Interactions between aroma and edible films. 1. Permeability Of methylcellulose and low-density polyethylene films to methyl ketones. *J Agric Food Chem, 47*(1), 108-113.

534 Sajilata, M. G., Savitha, K., Singhal, R. S., & Kanetkar, V. R. (2007). Scalping of Flavors in
535 Packaged Foods. *Comprehensive Reviews in Food Science and Food Safety, 6*(1), 17-35.
536 Salazar, R., Domenek, S., & Ducruet, V. (2014). Interactions of flavoured oil in-water
537 emulsions with polylactide. *Food Chemistry, 148*, 138-146.
538 Willige, Linssen, J., & Voragen, A. (2000). *Influence of food matrix on absorption of flavour*
539 *compounds by linear low-density polyethylene: Oil and real food products* (Vol. 80).
540 Woerdeman, D. L., Veraverbeke, W. S., Parnas, R. S., Johnson, D., Delcour, J. A., Verpoest,
541 I., & Plummer, C. J. G. (2004). Designing New Materials from Wheat Protein.
542 *Biomacromolecules, 5*(4), 1262-1269.
543 Zhou, Q., & Cadwallader, K. R. (2006). Effect of flavor compound chemical structure and
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

Figure captions

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

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

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

Table captions

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

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

Figure 1

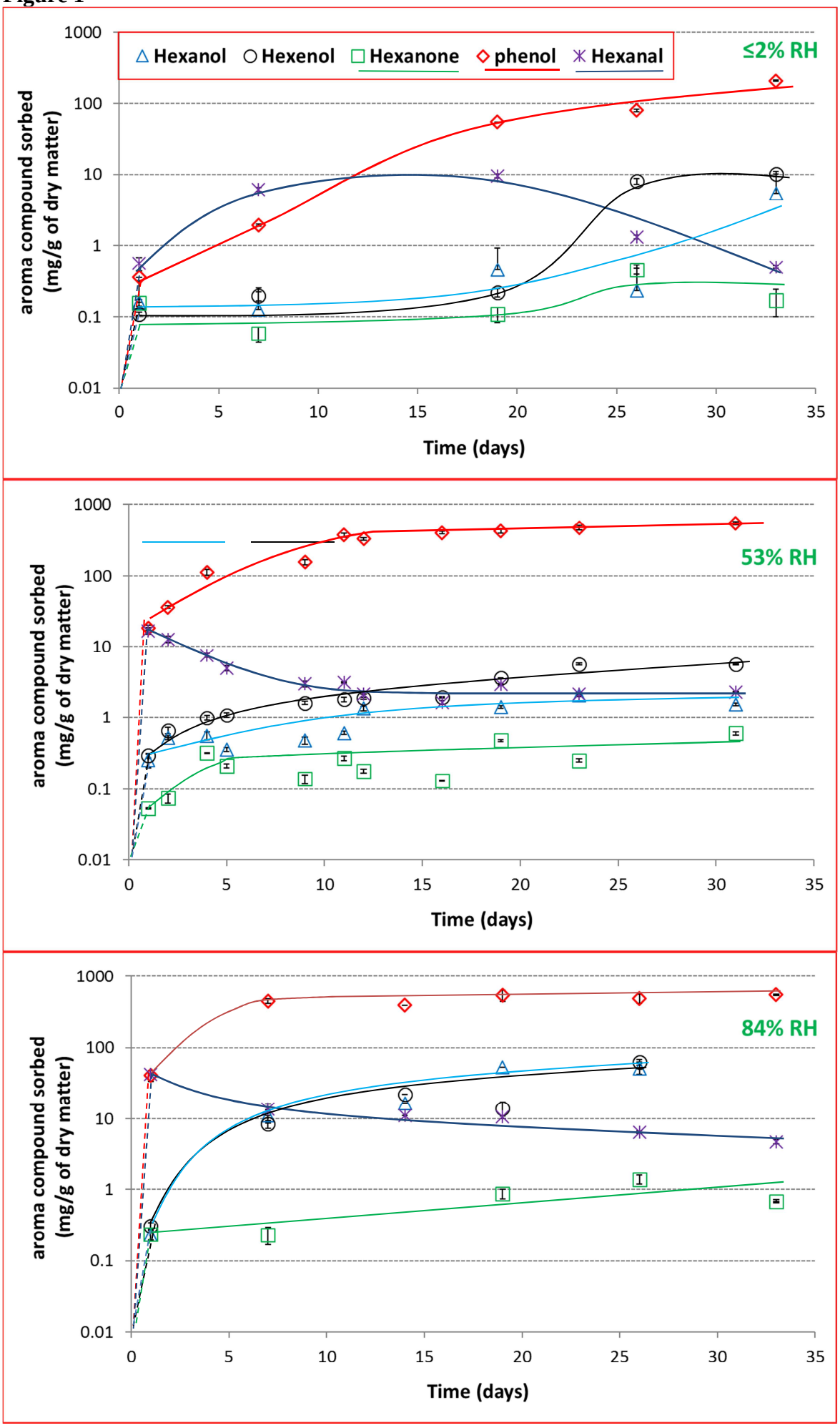
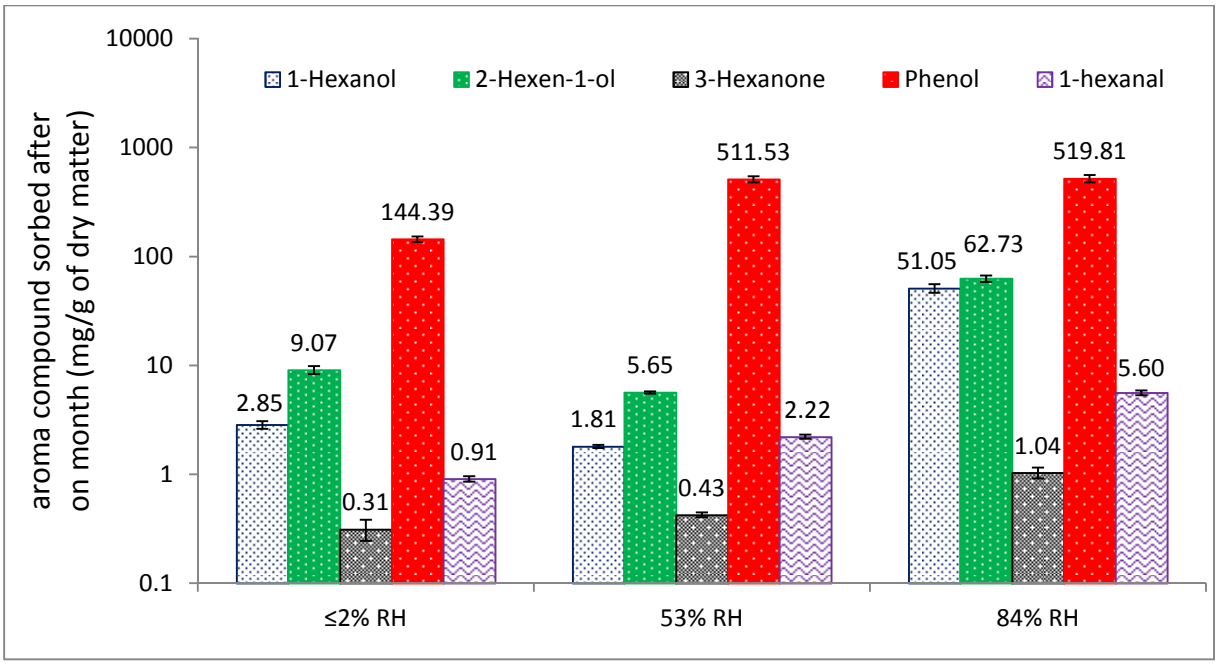
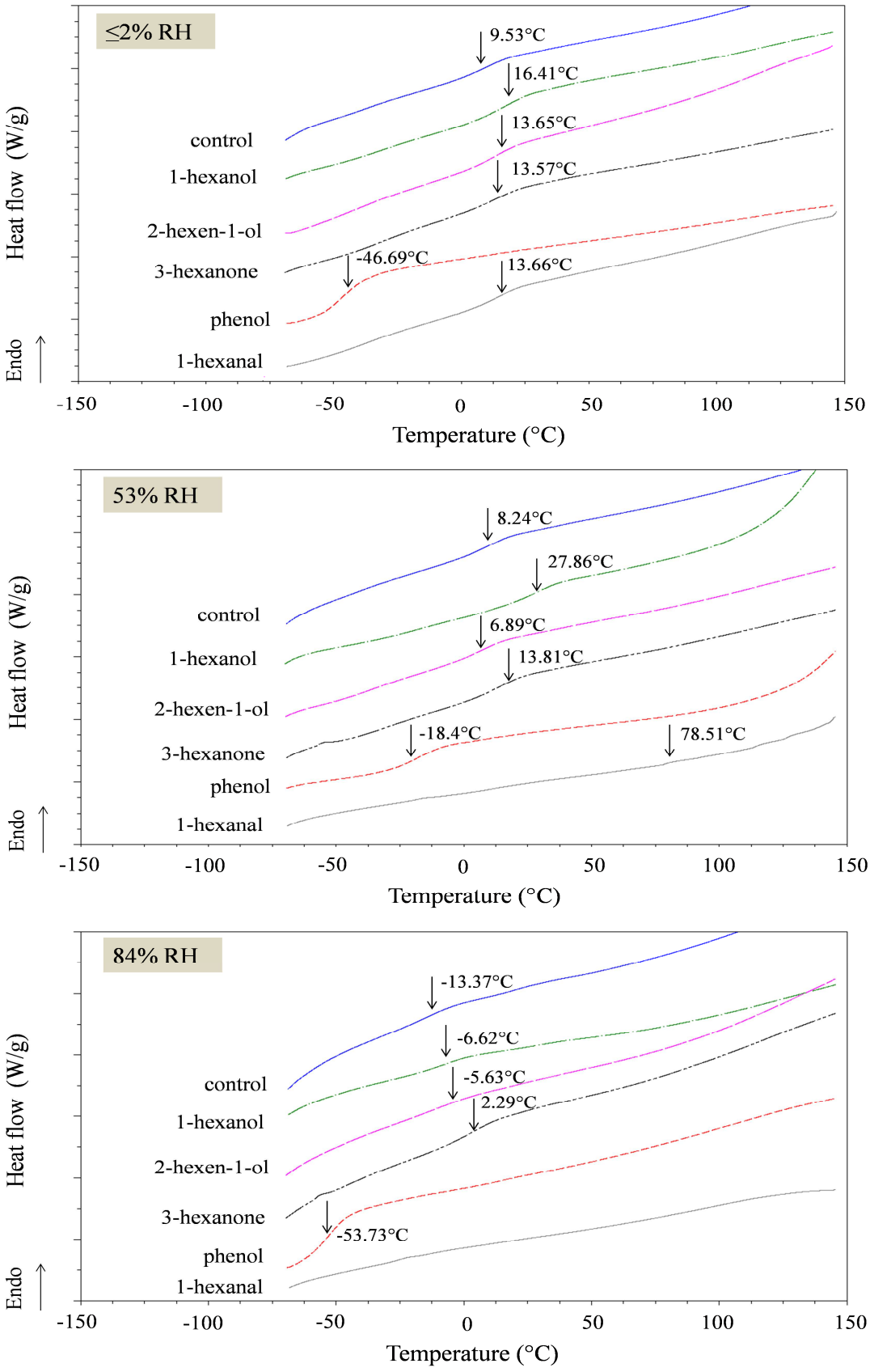

Hexanol
Hexenol
Hexanone
phenol
Hexanal
≤2% RH
53% RH
84% RH
aroma compound sorbed (mg/g of dry matter)
Time (days)
1000
100
10
1
0.1
0.01
0
5
10
15
20
25
30
35

Figure 2


aroma compound sorbed after on month (mg/g of dry matter)
10000
1000
100
10
1
0.1
1-Hexanol
2-Hexen-1-ol
3-Hexanone
Phenol
1-hexanal
2.85
9.07
0.31
144.39
0.91
1.81
5.65
0.43
511.53
2.22
51.05
62.73
1.04
519.81
5.60
≤2% RH
53% RH
84% RH

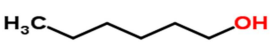
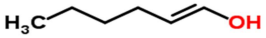
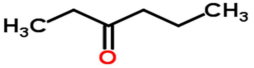
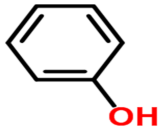
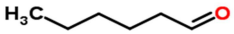
1 Figure 3


≤2% RH
9.53°C
16.41°C
13.65°C
13.57°C
-46.69°C
13.66°C
control
1-hexanol
2-hexen-1-ol
3-hexanone
phenol
1-hexanal
Heat flow (W/g)
Endo
-150
-100
-50
0
50
100
150
Temperature (°C)
53% RH
8.24°C
27.86°C
6.89°C
13.81°C
-18.4°C
78.51°C
control
1-hexanol
2-hexen-1-ol
3-hexanone
phenol
1-hexanal
Heat flow (W/g)
Endo
-150
-100
-50
0
50
100
150
Temperature (°C)
84% RH
-13.37°C
-6.62°C
-5.63°C
2.29°C
-53.73°C
control
1-hexanol
2-hexen-1-ol
3-hexanone
phenol
1-hexanal
Heat flow (W/g)
Endo
-150
-100
-50
0
50
100
150
Temperature (°C)

2

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

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

					
Molecular formula	$C_6H_{14}O$	$C_6H_{12}O$	$C_6H_{12}O$	C_6H_6O	$C_6H_{12}0$
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

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

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

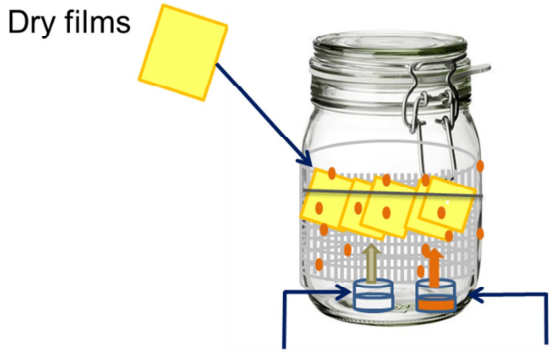
N.D. not detectable

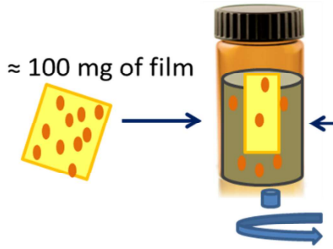
^{a,b,c,d,e,f,g,h}: values having the same letters for each parameter (Tg and Td) are not significantly different at p level =0.05

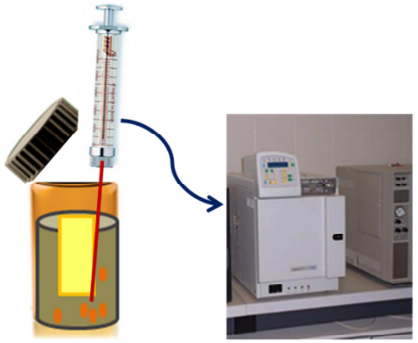
Sorption of the aroma compounds
Kinetic and equilibrium

Extraction of the aroma compounds

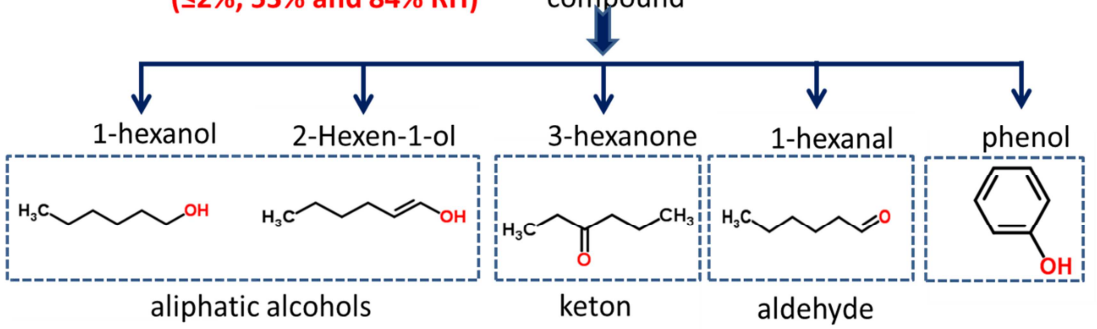
Quantification of the aroma sorption by GC



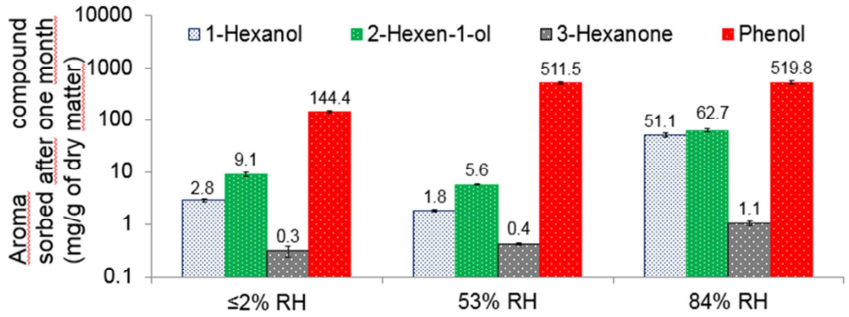




- **Yield of extraction ≥ 96%**
- **External calibration**



Quantity sorbed is moisture dependent



Film structure affected by sorbed aroma compounds