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How composition and process parameters affect volatile active compounds in biopolymer films

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ABSTRACT

Active edible films based on chitosan and containing an active aroma compound have been investigated. The influence of the composition of the film forming solution (solvent, plasticizers, nanoparticles, emulsifiers and aroma traps) and process parameters (temperature and viscosity) on drying kinetics, water content, colour, aroma compound retention and partition coefficient was studied. Solvent evaporation rate was increased by addition of ethanol. Water evaporation was delayed by glycerol. The aroma retention during film drying was directly related to the water content. The drying temperature had opposite influences according to the composition of the solvent and additives. Increasing temperature from 20 to 100 °C could increase 3 times the retention of aroma compounds. The viscosity of film forming solutions was strongly increased by biopolymer content and glycerol but reduced by carvacrol and ethanol. To improve carvacrol retention, the most efficient additive was gum arabic, followed by glycerol whereas the effect of nanoclays or emulsifiers remained weak.

1. Introduction

Dynamic changes in market trends and current consumer demands significantly increased the production of biopolymer active packaging materials. Active packaging is a type of packaging where product, packaging and surrounding environment interact in order to extend shelf life, improve organoleptic properties and/or food safety (Suppalku, Miltz, Sonneveld, & Bigger, 2003). The addition of an active substance in the material can improve packaging functionality (Appendini & Hotchkiss, 2002). There are several ways of its incorporation in the packaging system. One of the ideal ways of biopolymer packaging applications could be the incorporation of an active/antimicrobial compound directly within polymer matrix.

The effectiveness of antimicrobial films is determined by the release rate of the antimicrobial compounds, either directly to food or via air. Process conditions influence the final film structure and performances. Drying is generally accompanied by the loss of volatiles. Their retention during oven drying is affected by the nature of the matrix, the volatile concentration and the experimental conditions (e.g. drying rate, sample thickness, humidity) (Flink & Labuza, 1972). Carvacrol is a monoterpene phenolic constituent of essential oils with antimicrobial properties. Its retention and release depend on the polymer, the film preparation method, the interactions between polymeric and antimicrobial materials and environmental conditions (Cagri, Ustunol, & Ryser, 2004; Cha, Cooksey, Chinnan, & Park, 2003; Rico-Pena & Torres, 1991). Up to date, there are few studies dealing with the detection of the amount of the aroma compound in the film after processing and drying. Numerous authors have reported the initial amount of antimicrobial substances and not the real concentration in the dry films. Quantifying compounds mobility is a crucial element in understanding the mechanisms of release in the headspace or in a contact with food. Therefore, the retention of the active/antimicrobial compound is one of the most important features of active biopolymer film processing. Generally, when loss during drying is greater, the release in the vapour phase is faster. Sánchez-González, Cháfer, González-Martínez, Chiralt, and Desobry (2011) showed that limonene loss during drying ranged from 39 to 99% when added from 0.5 to 3% (w/w) in chitosan films.

Macromolecules such as chitosan show abilities to retain different antimicrobial substances. Chitosan (CS) offers real potential for applications in the food industry due to its particular physico-chemical properties, short time biodegradability, biocompatibility, antimicrobial and antifungal activities, non-toxicity, toughness, durability, moderate values of water and oxygen permeability (Agulló, Rodríguez, Ramos, & Albertengo, 2003; Bégin & Van

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Calsteren, 1999; Coma et al., 2002; Jeon, Kamil, & Shahidi, 2002; Muzzarelli et al., 1990; Ouattara, Simard, Piette, Bégin, & Holley, 2000; Shahidi, Arackchi, & Jeon, 1999; Wang, Du, Luo, Lin, & Kennedy, 2007). Drying methods, conditions and matrix formulation (e.g. polymer concentration, suitable solvent system, addition of plasticizers, dispersants, emulsifiers) have to be set up to engineer chitosan films likely to monitor the retention and thus the release of the active compound (Chillo et al., 2008; Fernández Cervera et al., 2004; Park, Marsh, & Rhim, 2002; Suyatma, Tightzert, & Copinet, 2005). The choice of the solvent in the preparation of a film forming solution is of a particular importance because it affects the solubility and dispersion of hydrophobic volatile molecules as well as the physico-chemical properties of the final products.

Polysaccharides are believed to induce the retention of aroma compounds caused either by molecular interaction with specific molecules or by an increase in viscosity of the matrix (Guichard, 2002). Changes in polymer concentration or mixing chitosan with other polymers may meet the above mentioned requirements when polymers are in solution. Arabic gum (AG) is a biocompatible and biodegradable polymer with more interaction sites and negative charges for interaction with polycationic polymers such as chitosan (Wareing, 1999). Because of its unique properties (e.g. emulsification, acid stability, low viscosity at high concentrations) it is used in many food applications such as beverages, emulsions, flavour encapsulation, as an agent that protects from oxidation and volatilization. The recovery and the oxidation stability of orange oil encapsulated in AG has been reported (Qi & Xu, 1999). Up to date, not a lot of work has been done to understand the aroma compound retention capacity of the chitosan blends with AG, particularly in dry matrices.

Emulsifiers are surface active molecules that interact simultaneously with polar and hydrophobic sites on the casting matrix, thus being able to improve the functional film properties (di Gioia & Guilbert, 1999). Both ionic and non-ionic surfactants, with or without plasticizer addition have been studied in different film productions (Andreuccetti et al., 2011; Bravin, Peressini, & Sensidoni, 2004; Morillon, Debeaufort, Blond, Capelle, & Voilley, 2002; Rodríguez, Osés, Ziani, & Maté, 2006; Villalobos, Hernández-Muñoz, & Chiralt, 2006). In an emulsion, droplets of aroma compound partition themselves among polymer matrix, according to their polarity and surface activity.

Montmorillonite (MMT) is a compound that can potentially be used to control the release of antimicrobial agents from film materials as displayed by Tunc and Duman (2010). Moreover, functional film properties were improved by the reinforcement of the polymer matrix with layered silicates (Park, Liang, Mohanty, Misra, & Drzal, 2004; Sothornwit, Rhim, & Hong, 2009; Tunc et al., 2007).

One way of investigating the retention (or release) of volatile compounds by polysaccharides is to measure the gas/matrix partition coefficient (*K*). This parameter describes the distribution of volatile compounds between the gas phase and the support matrix, after equilibrium is reached (Boland, Buhr, Giannouli, & van Ruth, 2004). Thus, a better understanding of this property is needed to adjust the volatile concentration in the packaging headspace. Several parameters should be taken into account. These include the type and the concentration of components along with the nature and the physicochemical properties of the aromatic compound (e.g. volatility and hydrophobicity) (Seuvre, Philippe, Rochard, & Voilley, 2006). According to these authors, polysaccharides influence the volatility of the molecules of the aroma compounds and their partitioning between different phases mainly by two mechanisms. The first one is diffusion decrease, predicted by the Stokes–Einstein equation where diffusion is inversely proportional to viscosity (Baines & Morris, 1987; Wilke & Chang, 1955). The second mechanism involves specific molecular interactions of the aroma compounds with the macromolecule. It is often due to adsorption, entrapment in microregions, complexation, encapsulation and hydrogen bonding (Godshall, 1997; Kinsella, 1989).

In this study, the aim was to investigate influence of drying parameters (temperature and relative humidity) and film formulation (polymer, casting solvent, and additives) on carvacrol retention and partition coefficient. The drying kinetics and some physical film properties were studied in order to develop chitosan films as vehicles for controlling aroma compound retention and release for further packaging applications.

2. Materials and methods

2.1. Materials and reagents

Commercial grade chitosan (CS) (France Chitine, Marseille, France, powder 652, having a molecular mass of 165 kDa, low viscosity, food grade, degree of deacetylation of 85%) and arabic gum (AG) (Spraygum, CNI, France) were used to constitute the film matrix. Anhydrous glycerol (Fluka, 98% purity, Fluka Chemical, Germany), Tween 20 (Sigma–Aldrich), lecithin (Sigma–Aldrich), nanoclays (montmorillonite, synthesis and characterization is detailed elsewhere, Reinholdt, Miehé-Brendlé, Delmotte, Le Dred, & Tuilier, 2005), polyethylene glycol (PEG750, Aldrich Chemicals) and glycerol monostearate (Sigma–Aldrich) were used in order to improve aroma compound retention and mechanical properties. Acetic acid (glacial 100%, Merck, Darmstadt, Germany) and pure ethanol (absolute, Sigma–Aldrich) were used as solvents in the preparation of the film forming solutions (FFS). All the chemicals were used without further purification and freshly prepared solutions were used in all experiments. Carvacrol with a purity of about 97% (Fluka) was used as a model antimicrobial volatile compound. *n*-Hexane (Chromasolv, purity of >97%, Sigma–Aldrich) was used as an extraction solvent.

2.2. Film formation

2.2.1. Chitosan films (CS)

A chitosan solution was prepared by dissolving the chitosan powder in the 1% (v/v) aqueous acetic acid, to obtain 1, 2 or 3% (w/v) solutions. To achieve the complete dispersion of chitosan, the solution was stirred for 2 h at room temperature. To prepare aqueous hydroalcoholic acid media, ethanol was mixed in ratio ethanol:aqueous acetic acid 30:70. Different amounts of glycerol (up to 50%, w/polymer dry matter (p.d.m.)) were added to the chitosan solution under stirring. The aroma compound (from 0.01 to 3%, w/v) was dispersed in the film forming solution (FFS) and the mixture was homogenized at 24,000 rpm for 10 min with an Ultra Turrax (T25 IKA). FFS was then poured into a glass Petri dish. In order to obtain films, solvents were removed by drying in a ventilated climatic chamber (KBF 240 Binder, ODIL, France) at fixed temperatures ranging from 20 °C to 100 °C and RH ranging from 10 to 80%. After drying, the films were peeled off the surface and stored in a ventilated climatic chamber (KBF 240 Binder, ODIL, France) before measurements. The aroma compound-free films were prepared using the same procedure, instead of the step where the aroma compound was added. PEG (up to 30%, w/p.d.m.), lecithine (from 0.5 to 30%, w/p.d.m.), Tween 20 (from 2.5 to 30%, w/p.d.m.) and glycerol monostearate (up to 5%, w/p.d.m.) were added in some film formulations after solubilisation of chitosan powder.

2.2.2. Chitosan/arabic gum blends (CS/AG)

To achieve CS/AG composite films, AG was added in the FFS of CS (50%, w/w) and mixed under magnetical stirring for 2 h. The next steps were the same as for the CS film.

2.2.3. Films with nanoclays (CS/NC)

In order to study whether NC addition has an impact on the aroma compound retention and release properties of the dried films, CS/NC films were prepared. First, CS was dispersed in the 1% (v/v) aqueous acetic acid, to obtain 1, 2, or 3% (w/v) solutions. Meanwhile, an exact amount of NC powder (to obtain 10%, w/w dry polymer) was dispersed into 30 mL of distilled water under magnetic stirring at 500 rpm for 2 h. In a second step, NC suspension was added to the film forming solution under mixing at 5000 rpm for 15 min. In the last step, carvacrol was added, and the mixture was homogenized for 10 min at 24,000 rpm with an Ultra Turrax (T25 IKA). As nanoclays are aimed to induce the brittleness of dry films, glycerol was added in order to improve the film mechanical properties. Actually, two different sequences, differing in order of addition of the NC were prepared. In the first sequence, named A, after CS dissolution, glycerol was added (30, 40 or 50, w/dry polymer matter) and FFS was mixed next 15 min. Meanwhile, carvacrol was mixed with NC dispersion. For the second sequence, named B, glycerol was added to clay suspension under mixing for 10 min. Then, two suspensions were mixed together for 15 min at 5000 rpm. In the last step carvacrol was added to the mixture and following procedure used was as the one used for all other films.

2.3. Rheological behaviour of film forming solutions

The rheological behaviour of FFS was analyzed at 20 ± 1 °C by means of a viscosimeter (HAAKE RotoVisco 1, Thermo Electron Corporation, Karlsruhe, Germany). Rheological curves were obtained after a stabilization time of 3 min. The shear stress τ was measured as a function of shear rate γ (s^{-1}) from 0 to 250 s^{-1}. The power law model:

$$\tau = K \times \gamma^n$$

was applied to determine the consistency index (K, Pa/s) and the flow behaviour index (n, dimensionless).

2.4. Film characterizations

Before characterization, all the film samples were conditioned at 23 °C and 30% RH up to equilibrium.

2.4.1. Film thickness

Film thickness was measured with an electronic gauge (PosiTector 6000, DeFelsko Corporation, USA). The final values were reported as the mean of 3 measurements at 3 different locations of the film surface.

2.4.2. Aroma compound retention in biopolymer matrix

In order to follow the aroma compound release/retention, an extraction technique and a gas chromatography analysis were performed. *n*-Hexane was used as an extraction solvent (due to high affinity of carvacrol for *n*-hexane). Slices of dry film were put in the glass vial and a known amount of *n*-hexane was added at ratio 0.2:1.5, followed by stirring till all the aroma compound was extracted. The aroma compound extraction yield was taken into account for the final calculation. The extraction yield was measured by successive extractions till the sample was exhausted.

The aroma compound was quantified by injecting the extract into a gas chromatograph and calculated from an external calibration curve. For each sample three repetitions were performed.

2.4.3. Measuring of the aroma compound in gas phase

In order to measure the carvacrol release from the film (solid phase) into a headspace (gas phase), a static headspace method was used. The film was put into a headspace vial (40 mL Supelco) and then sealed immediately with Mininert Valves (Supelco). Each measurement was carried out at the equilibrium and the time determined to reach equilibrium was 4 h at 25 °C. After equilibration, a volume of 1 mL of headspace air was taken out using a gastight syringe (Hamilton, Switzerland) and injected in the gas chromatograph. Two headspace injections were made per vial. The amount of carvacrol released in the gas phase was calculated from an external calibration curve.

2.4.4. Gas chromatography analysis

The volatile flavour compounds in the film extracts and in the headspace above the film were analyzed by a Shimadzu GC 14B gas chromatograph, equipped with a flame ionization detector (GC-FID) and 30 m length DB-Wax column (J&W) with 0.53 mm i.d. and 1.0 μm film thickness and nitrogen as carrier gas (60 kPa). Hydrogen and air were used as ignition gases. The oven temperature programme was set at 210 °C, isothermal. The injector and detector temperature were at 240 °C.

2.4.5. Calculation of partition coefficient

The partition coefficient is defined as the mass concentration ratio of the molecule between two phases. After drying, the obtained antimicrobial film is a solid matrix. The partition coefficient studied in this work can be defined as follows:

$$K = \frac{C_g}{C_f}$$

with C_g, concentration of the volatile compound in the gas phase ($g_{\text{aroma}}/g_{\text{air}}$) above the C_f, concentration of the volatile compound in the film ($g_{\text{aroma}}/g_{\text{film}}$).

2.4.6. Colour measurement

The colour of the film was determined using a colorimeter (Minolta, CM-3600d, Tokyo, Japan). Hunter L^*, a^*, and b^* values were averaged from three readings across for each sample, and then the total colour difference (ΔE) was calculated according to Ghorpade, Li, Gennadios, and Hanna (1995).

2.4.7. Determination of drying curves

Drying curves were obtained by plotting a graph of moisture ratio versus time. The moisture content at each time interval (once per hour) was calculated from the weight loss data and the dry solid weight of the sample, till no further weight loss could be measured. Aroma compound concentration in solution/gel/film at a given time was determined as described in Section 2.4.2.

2.5. Statistical analysis

The statistical analysis of data was performed through variance analysis (ANOVA) using Xlstat-Pro (win) 7.5.3. (Addinsoft, New York). The data were ranked and statistical differences were evaluated on the ranks with a one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests. The principal component analysis (PCA) was used for detailed examination of the data. In all cases, a value of $p < 0.05$ was considered to be significant.

3. Results and discussion

3.1. Characterization of the film forming solutions

Viscosity can influence drying kinetics and aroma compound retention. The experimental flow curves of film forming solutions (FFS) between 0 and 250 s^{-1} (Fig. 1) have been very closely fitted by Ostwald de Waale model ($r > 0.99$). The addition of ethanol changed the solution rheological properties. Hydroalcoholic acid solutions showed more pronounced pseudoplastic behaviour (n value from

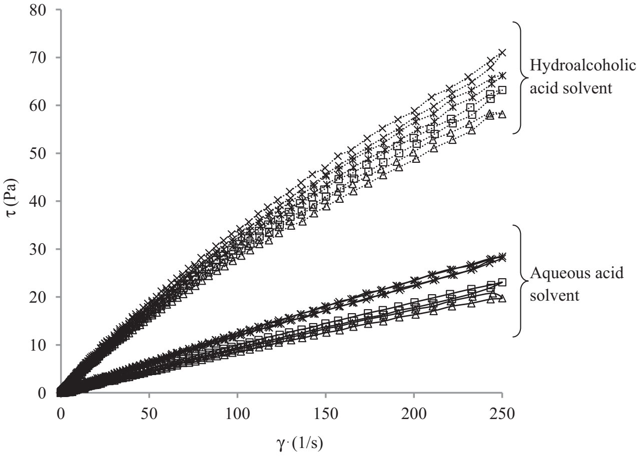

Fig. 1. Typical flow curves, at 20 °C, of the film forming solutions prepared in the acidic media (continuous lines) or in the alcoholic media (dashed lines). ×, chitosan; Δ, chitosan and carvacrol; □, chitosan, carvacrol and glycerol; ✳ chitosan and glycerol.

0.82 to 0.84), whereas a less shear thinning behaviour was observed for aqeous acidic media (*n* value from 0.93 to 0.95). A viscosity decrease attributed to the conformational change of the macromolecular chains was reported (Bonilla, Atarés, Vargas, & Chiralt, 2012; Kasaai, Charlet, Paquin, & Arul, 2003; Vargas, Perdones, Chiralt, Cháfer, & González-Martínez, 2011). Higher alcohol and chitosan content resulted in greater viscosity. Solvent polarity changed the organisation of chitosan chains and therefore viscosity increased. In more concentrated hydroalcoholic acidic FFS structuring degree was greater and therefore dynamic viscosity too. Those associated structures formed macro clusters in concentrated polymer solutions (Uspenskii, Vikhoreva, Sonina, & Gal'braikh, 2010).

The incorporation of carvacrol, as a non miscible compound, significantly reduced viscosity in both systems: from 173 to 111 mPa/s and from 683 to 633 mPa/s for aqueous acidic and hydroalcoholic FFS, respectively. Indeed, the adsorption of polymer on the droplet surface favours a decrease of its effective thicknening concentration in the aqueous media (Bonilla et al., 2012). In aqeous acid solutions glycerol interfered with the polymer chains, and provoked a decrease in chitosan viscosity. Therefore *K* values increased (from 0.11 to 0.13). This effect was not observed in the hydroalcoholic solutions. Different solvents probably affect the drying and the final chitosan film structure in the same way as FFS properties which govern film functional properties.

3.2. Drying process

Drying is considered as one of the most important steps during biopolymer film preparation (Arvanitoyannis, Nakayamab, & Aibab, 1998; Kaya & Kaya, 2000; Sothornvit & Krochta, 2005). During drying, all volatile agents, including solvents and aromas are progressively evaporated. Consequently, as solvent removal increases, the film dry matter increases too. Increasing drying temperature boosts the volatilization of the volatile compounds present in the film forming structure. Thus drying time was shorter (Tables 1 and 2). Drying temperatures seemed to be an important factor influencing both physico-chemical film properties and carvacrol retention.

Hydrophobic carvacrol is naturally immiscible in hydrophilic biopolymer solutions. Ethanol was used to enhance carvacrol dissolution, to obtain better mixing with aqueous acetic acid and to decrease drying time. After ethanol is evaporated, the remaining system is a hydrophilic film containing hydrophobic substance. It is important to realize that, naturally, such a system has a limited uptake. Carvacrol was unavoidably evaporated with solvents. But, the overall mechanism of aroma compound loss is complex because there is simultaneously a change in the biopolymer viscosity, a change in the physical state from liquid to solid, a change in polarity due to the differential evaporation rate between volatiles, a probable change in the interaction nature and strength, and finally a change in the macromolecular structure as here after suggested.

In the crystal, chitosan chains are packed in an antiparallel way. In the salt solutions with acetic acid, called Type II salts, the chitosan molecule takes up a relaxed two fold helix composed of asymmetric units of tetrasaccharide. Since water molecules are included in the crystal, they tend to enter between these sheets and stabilize the crystal structure. Type II crystals change to the annealed chitosan polymorph by spontaneous water-removing action of the acid (Ogawa, Yui, & Okuyama, 2004). Amorphous structures that might be obtained by solvent casting greatly contributed to the matrix relaxation and carvacrol release. The polarity of hydrophobic carvacrol molecules was changed after solubilisation and dispersion in hydroalcoholic FFS. Thus, more molecules were locked in the chitosan network. Solute polarity is an important factor in transfer process (Arora, Hansen, & Armagost, 1991; Matsui, Ono, Shimoda, & Osajima, 1992). Therefore, as ethanol evaporates faster than water, in the beginning of the drying, carvacrol losses were more pronounced.

Moisture and carvacrol content in the sample in different intervals were determined (Fig. 2). The time required to obtain constant film weight for hydroalcoholic and aqueous acidic solutions was 6 and 7 h, respectively (Fig. 3). To ensure that weight changes were negligible, films were left in the drying chamber up to 14 h. Generally, the loss of volatiles was greatly influenced by the water content in drying film (Fig. 3). Therefore, there was no significant change in carvacrol content when films were left up to 14 h. Mayachiev and Devahastin (2010) reported that at ambient temperature it took 52, 50 and 47 h for 1, 2 or 3% Indian gooseberry extract to obtain dry films, respectively.

Theoretically, heat and mass transfer have a constant rate at the beginning and they slow down after reaching a critical moisture content. At high moisture levels, the carvacrol rate loss from a drying solution is mainly dependent on its volatility. Higher water uptake resulted in matrix plasticization and then its release was facilitated. Soottitantawat et al. (2005) reported that the release rate of menthol increased, upon increasing water activity. Glycerol attracts and deters water molecules from changing the shape of the drying curves. The evaporation rate is proportional to the saturated water vapour pressure above the solution surface. This pressure is considered as constant throughout the drying process until the final stages, when the pressure is reduced. On the contrary, curves do

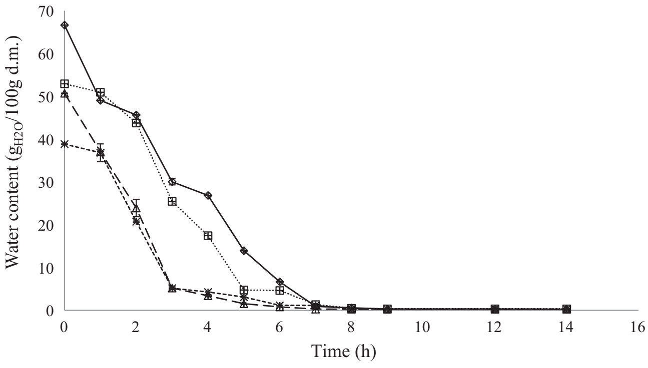

Fig. 2. Drying curves of the chitosan solutions. ◇, chitosan solubilised in acidic media; □, chitosan solubilised in the acidic media with glycerol; Δ, chitosan solubilised in the alcoholic media; ×, chitosan solubilised in the alcoholic media with glycerol.

Table 1
Colour parameters of chitosan films influenced by the composition. Samples were dried at 20 °C and 30% RH.

Film	Chitosan dry matter (g)	Glycerol % (w/p.d.m.)	Nanoclays or arabic gum % (w/p.d.m.)	Drying time (h)	L^*	a^*	b^*	ΔE
Chitosan film without carvacrol	1	0	0	14	96.40	-0.97^{c}	4.40^{g}	2.64^{h}
	2	0	0	14	96.30^{b}	$-1.23^{c,d}$	5.00^{g}	3.29^{h}
	3	0	0	18	$96.10^{b,c}$	$-1.37^{c,d}$	5.23^{g}	3.55^{h}
Chitosan film with carvacrol	1	0	0	14	97.57^{a}	$-0.93^{c,d,e}$	5.03^{g}	5.59^{g}
	2	0	0	14	96.33^{b}	$-1.23^{c,d}$	5.27^{g}	5.81^{g}
	3	0	0	18	94.90^{d}	$-1.27^{c,d}$	$5.60^{f,g}$	$6.13^{f,g}$
Chitosan film with carvacrol glycerol	1	30	0	14	94.07^{d}	$-1.73^{d,e,f}$	14.20^{d}	12.43^{d}
	2	30	0	14	88.37^{g}	$-1.80^{d,e,f}$	23.97^{b}	22.16^{b}
	3	30	0	18	94.43^{d}	$-1.77^{d,e,f}$	14.43^{d}	12.66^{d}
Chitosan film with carvacrol and nanoclays	1	0	10	12	NA	NA	NA	NA
	2	0	10	12	90.37^{f}	-0.83^{c}	16.47^{c}	16.8^{c}
	3	0	10	13	NA	NA	NA	NA
Chitosan film with carvacrol, glycerol and nanoclays	1	30	10	14	89.00^{g}	-2.17^{f}	26.53^{a}	26.83^{a}
	2	30	10	14	85.00^{h}	0.67^{b}	23.80^{b}	24.11^{b}
	3	30	10	16	85.37^{h}	0.43^{b}	23.57^{b}	23.87^{b}
Chitosan/arabic gum blends	3	30	50	12	91.70^{e}	7.20^{a}	17.00^{c}	16.86^{c}
	3	40	50	12	$95.07^{c,d}$	$-1.93^{e,f}$	9.70^{e}	10.11^{e}
	3	50	50	13	$96.63^{a,b}$	$-1.93^{e,f}$	7.47^{f}	7.92^{f}

NA, not measured because produced films were too brittle. Different superscripts (a–h) within a column indicate significant differences among formulations ($p < 0.05$).

not display a linear trend (Figs. 2 and 3). Due to the internal heating phenomenon, saturated pressures became larger and thus led to non-linear slopes of hydroalcoholic and aqueous acid FFS curves, respectively. Over the time, structural change in the thin layer of FFS modifies diffusion which disturbs the evaporation rate. According to Buonocore, Del Nobile, Panizza, Corbo, and Nicolais (2003) the release of an active compound from the polymer network took place in several steps. Correspondingly, carvacrol loss depended on intermolecular migrations between chitosan chains, its solubility and its diffusion towards the matrix surface. During the first hour, less aroma compound was released from plasticized FFS. The water evaporation rapid rate was delayed for about 2 h (Fig. 3C and D). At the end, glycerol did not have any significant effect on the overall drying period. The gelatinization technique and the drying method influenced the tapioca starch/glycerol network characteristics and caused changes in physical film properties (Flores, Haedo, Campos, & Gerschenson, 2007). Below gelation point, more than 50% of initially added carvacrol was lost (Fig. 3). These values correspond to ~10% of water in sol/gel. Indeed the authors reported that at gel point, the water content was below 15% (Batista, Rosa, & Pinto, 2007). Once dried, the plasticized chitosan films had more water than the unplasticized ones. Similar results were mentioned by Ziani, Oses, Coma, and Maté (2008). At low moisture contents the diffusion coefficient became a determining loss factor.

3.3. *Film macroscopic appearance*

The film appearance was studied by colour measurement according to Ghorpade et al. (1995) and was done for the targeted application and not for the physico-chemical reactivity. Film transparency is a key to good film acceptance by users. Therefore, two different aspects of colour changes have been measured as a function of the composition and the drying temperature and results are given in Tables 1 and 2.

CS and CS/carvacrol films presented good transparency as indicated by high lightness values (L^*) that ranged from 94.9 to 96.4 (Tables 1 and 2). A significant decrease in this parameter was found when both glycerol and NC were incorporated (85.4, 85.0 and 89.0 for CS 3, 2, or 1% (w/v) respectively). L^* was higher than that of chitosan-based nanocomposites (66.8 and 85.8 (Rhim, Hong, Park, & Ng, 2006). It was probably because of different types of NC. Still it was in the same range as obtained by Casariego et al. (2009). Martins, Cerqueira, and Vicente (2010), found that the incorporation of α-tocopherol on chitosan based films led to a decrease

Table 2
Influence of drying temperature on the colour parameters and the drying time of the chitosan based films. All films were plasticized with glycerol (30%, w/p.d.m.) and dried at 30% RH.

Film	Drying temperature (°C)	Drying time (h)	L^*	a^*	b^*	ΔE
Chitosan 1% (w/p.d.m.)	20	14	94.07^{b}	-1.73^{f}	14.20^{g}	12.42^{h}
	60	1.75	94.07^{b}	-1.50^{f}	13.30^{g}	11.50^{h}
	100	0.75	94.13^{b}	-1.53^{f}	13.20^{g}	11.41^{h}
Chitosan 2% (w/p.d.m.)	20	14	88.37^{d}	-1.80^{f}	23.97^{d}	$22.16^{d,e}$
	60	4.5	86.33^{e}	-1.63^{f}	$22.07^{d,e}$	$20.25^{e,f}$
	100	0.75	84.37^{f}	-1.33^{f}	20.13^{e}	$18.30^{f,g}$
Chitosan 3% (w/p.d.m.)	20	18	$94.43^{a,b}$	-1.77^{f}	14.43^{g}	12.66^{h}
	60	1.75	96.30^{a}	-1.33^{f}	7.23^{h}	5.48^{i}
	80	1.5	96.17^{a}	-1.33^{f}	6.83^{h}	7.31^{i}
	100	0.75	96.23^{a}	-1.40^{f}	6.50^{h}	4.78^{i}
Chitosan/arabic gum blends (50:50, w/w)	20	12	91.70^{c}	7.20^{d}	17.00^{f}	23.09^{d}
	60	3.5	75.27^{g}	7.60^{d}	49.07^{b}	47.85^{b}
	80	1	71.40^{h}	10.67^{c}	57.50^{a}	56.69^{a}
	100	0.5	45.57^{j}	21.53^{a}	27.33^{c}	33.50^{c}
Chitosan 3% (w/p.d.m.) with nanoclays (10%, w/p.d.m.)	20	14	$85.00^{e,f}$	0.67^{e}	23.80^{d}	24.11^{d}
	60	3	$85.37^{e,f}$	0.43^{e}	23.57^{d}	23.87^{d}
	100	0.75	63.97^{i}	14.37^{b}	47.57^{b}	47.84^{b}

Different superscripts (a–i) within a column indicate significant differences among formulations ($p < 0.05$).

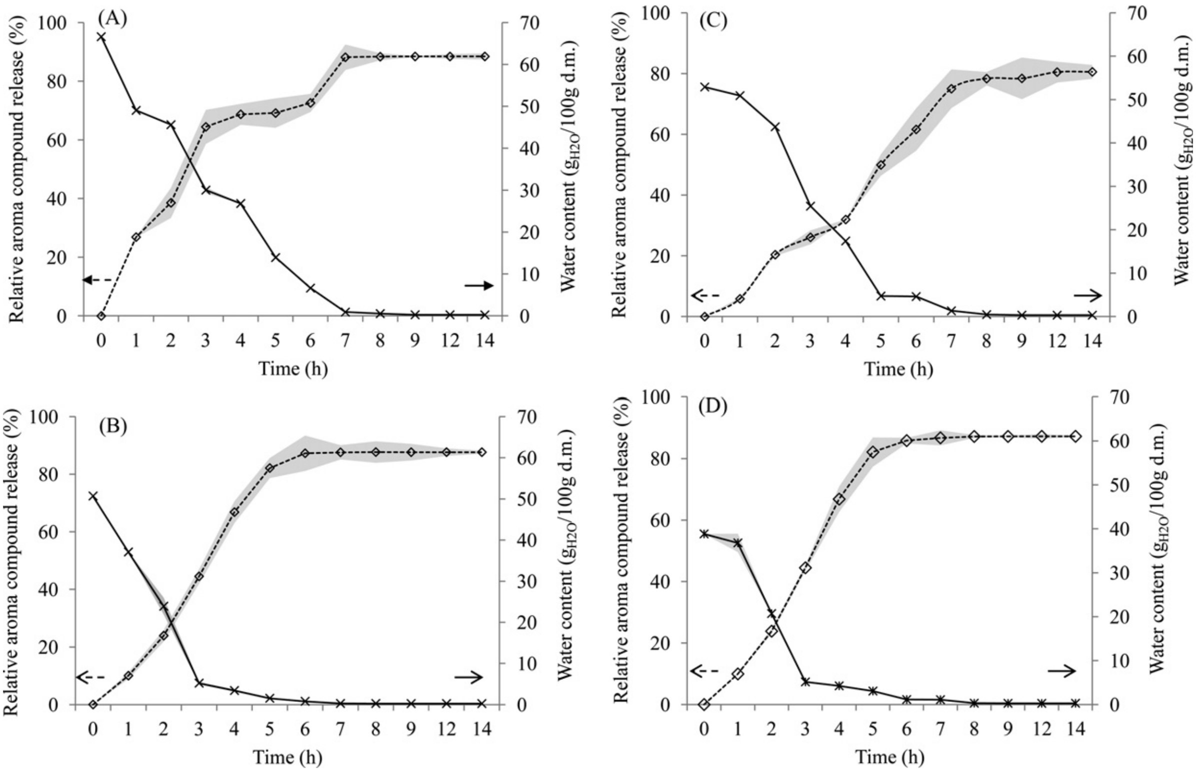

Fig. 3. Water content (g_{H_2O}/100 g d.m.) and relative carvacrol release (% of initially added amount) from chitosan based films during drying. (A) Aqueous acidic media, (B) hydroalcoholic media, (C) aqueous acidic media with glycerol and (D) hydroalcoholic media with glycerol. (The grey zones correspond to standard deviation of the curves.)

in lightness (from 95.4 to 92.3) and an increase in greenness (a^* decreased from 3.54 to −1.42). The b^* value is the parameter describing the natural colour of chitosan, and it was the chromatic coordinate that influenced the total colour difference. b^* increased from 5.94 to 15.73 and it indicated the yellowness of the CS films. The yellowness was intensified with CS, glycerol and AG addition.

As an indicator of global colour changes, ΔE was calculated from other colour parameters. b^* and ΔE increase in the presence of NC was previously reported (Casariego et al., 2009; Park et al., 2002). Again, this value showed more changes with increasing temperature, especially when CS was blended with AG. Srinivasa, Ramesh, Kumar, and Tharanthan (2004), observed an increase in yellowness in oven-dried chitosan films. Furthermore, when the casting temperature was increased, the films presented different yellow and green colour changes (lower b^* and a^*). These changes may be attributed to oxidation, nonenzymatic browning or Maillard reaction between protein traces and reducing sugars from AG but no chemical analysis has been done to confirm this hypothesis. It was reported that glycerol in chitosan may be oxidized and the products could react with chitosan through the Maillard reaction according to Deng et al. (2009) and Kanatt, Chander, and Sharma (2008). In Tables 1 and 2 decrease in L^* value at higher temperatures can be due to the presence of glycerol and not NC. L^* changed remarkably for CS/AG blends (75.3, 71.4 and 45.6 for 60, 80 and 100 °C respectively) and films with NC (85.4 and 63.9 for 60 and 100 °C, respectively). Overall colour changes were more intense with higher amounts of acetic acid for chitosonium acetate films heated for 2 h at 120 °C (Kam, Khor, & Lim, 1999).

3.4. Film properties as a function of composition and drying conditions

3.4.1. Influence on mass partition coefficient

Usually, the mass partition coefficient K_{mass} (ratio between the aroma compound concentration in the air and the aroma compound concentration in the film) is inversely proportional to the retention capacity during processing. K_{mass} can be related to carvacrol retention by chitosan based films during film processing (casting plus drying) (Fig. 4). Actually, more than 200 formulations in drying temperature interval from 20 to 100 °C and various humidities (from 10 to 80% RH) were tested. Formulations included two polymers (chitosan and arabic gum) and various additives (at various concentrations) as aroma compound retention enhancers such as glycerol, polyethylene glycol, Tween 20, lecithin and nanoclays. The partition coefficient of an aroma compound between the headspace and the film matrix is influenced by many factors (e.g. volatility, polarity, water solubility and temperature) (Bangs & Reineccius, 1982; Saravacos & Moyer, 1968). The composition, the physico-chemical properties of the matrix as well as the intra-network interactions can change the thermodynamic behaviour of volatile molecule reflecting its ability to reach a gaseous phase. Only free dissolved molecules exert a vapour pressure and therefore, they can influence K_{mass} (de Roos, 2003). The principal component analysis (PCA) was done to better identify the main parameters of the composition/process which

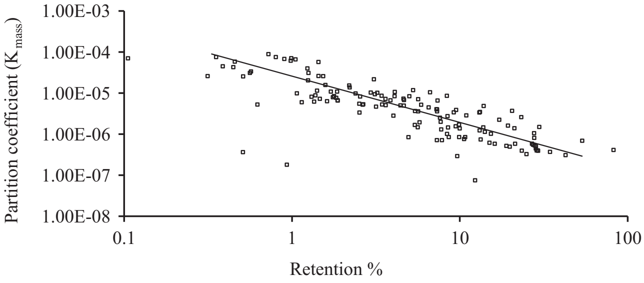

Fig. 4. Relationship between the carvacrol retention during the film processing and the air/film partition coefficient during the film storage.

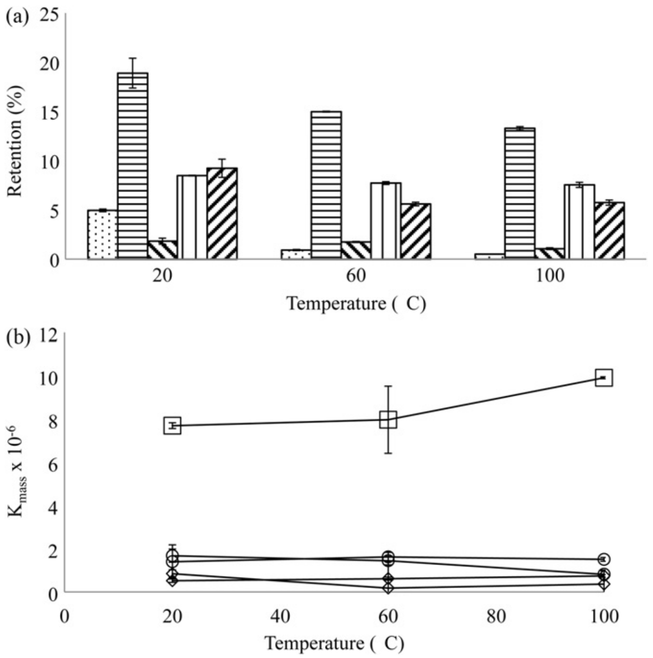

Fig. 5. Carvacrol retention and the air/biopolymer partition coefficient (K_{mass}) in the chitosan based films with or without the addition of nanoclays when dried at temperature scale from 20 to 100 °C. (A) carvacrol retention: ⬚, CS 1% (w/v), ▤, CS 2% (w/v), ▥, CS 3% (w/v), ▨, CS/NC_A, ▧, CS/NC_B; (B) partition coefficient: ○, CS 1% (w/v), △, CS 2% (w/v), □, CS 3% (w/v), ✳, CS/NC_A, ◊, CS/NC_B.

influenced carvacrol retention, drying time and K_{mass}. It appeared that carvacrol retention in chitosan based films increased with decreasing drying time, drying humidity and NC (axis representation and data of the PCA analysis not given). A negative correlation was found for CS, glycerol and AG addition, drying temperature and dry film thickness. The time required to obtain dry films depended mostly on film thickness, CS, AG and glycerol. With temperature increase, drying time was shorter. Besides, K_{mass} increased with drying temperature and humidity increase, whereas it decreased when more CS, glycerol or AG were present in thicker films.

Higher chitosan concentrations did not show significant differences in K_{mass}, being in order of magnitude of 10^{-6} (3.88×10^{-6}–6.18×10^{-6}). Addition of glycerol lowered carvacrol release from dry CS films whereas K_{mass} was in order of 10^{-7}. On the contrary, the rise in glycerol/polymer dry matter ratio in CS/AG blends, facilitated carvacrol release, and therefore K_{mass} increased up to 10^{-5}. The protective colloid functionality of AG led to lower partitioning of the aroma compound between films and surrounding atmosphere. Even though better protection with AG during drying was observed, in equilibrium, the amount of released carvacrol from dry film remained equal (data not shown). Consequently, no significant difference in K_{mass} was observed. The effect of temperature on the K was previously studied by Ettre and Kolb (1991), Kolb, Welter, and Bichler (1992) and Carelli, Crapiste, and Lozano (1991). According to literature, the relationship between $\log K$ and $1/T$ may be considered as linear. Temperature increases the aroma compound volatility and the diffusivity according to Henry's law (Buttery, Bomben, Gaudagni, & Ling, 1971). Therefore at higher temperatures the release of volatile is higher and K_{mass} lower. The final result is less aroma compound (Fig. 5A). This behaviour was confirmed for CS films, but not for CS/AG blends. The possible explanation is better stability of AG/carvacrol complexes formed in CS/AG blends at higher temperatures, as will be discussed further on.

Finally, to better understand carvacrol retention behaviour, the most important parameters (CS dry matter, glycerol, NC and GA) were studied. Furthermore the influence of the drying temperature was discussed.

3.4.2. *Influence of chitosan concentration*

Different concentrations of chitosan in film forming solutions changed structure, thickness and carvacrol retention. Generally, a lower amount of polymer indicates a reduction in its retention capacity. It was attributed to the inclusion of carvacrol molecules within the matrix. This phenomenon was explained by Voilley and Simatos (1980). A sol/gel transition occurs faster when the chitosan solution is more concentrated (Montembault, Viton, & Domard, 2005). Then again, 1% (w/v) chitosan FFS remained aqueous for a longer period and guided with solvent evaporation more carvacrol was lost. On the other hand Boland et al. (2004) reported a higher release with an increased matrix rigidity. This might explain why films with the highest chitosan concentration entrapped a lesser quantity of aroma compound. At higher temperatures, polymer concentration plays an important role. Retention varied from 4.9 to 18.9, from 0.93 to 14.99 and from 0.51 to 13.29 for 1 and 2% CS films dried at 20, 60 and 100 °C, respectively. Several explanations could be made. First of all, it was expected that a higher carvacrol loss would occur at temperatures close to its flash point (106 °C). Drying at lower temperatures does not only decrease aroma compound volatilization, but it might also influence polymer crystallinity. Srinivasa et al. (2004) stated out that ambient dried chitosan films had a lower crystallinity than those at 70, 80 or 90 °C. There are two main explanations of interactions between polysaccharide and aroma molecules (viscosity modifications and molecular interactions (hydrophobic interactions, hydrogen and covalent bonding)) (Baines & Morris, 1987; Lubbers, Landy, & Voilley, 1998; Roberts, Stephen Elmore, Langley, & Bakker, 1996; Secouard, Malhiac, Grisel, & Decroix, 2003). In theory, hydrophobe/hydrophobe interactions are endothermic reactions, therefore an increase in temperature amplifies them. This explains why chitosan macromolecules were networked faster at higher temperatures, forming a denser structure and leaving fewer spaces for carvacrol.

Generally, the viscosity increased when the concentration of the dispersed phase increased. Increased temperature can increase flexibility of chitosan macromolecules, and as a result, viscosity decreases. Therefore, films cast from less dense chitosan solutions lost more volatiles. The authors reported that highly volatile aroma components are much better retained with an increase in the feed solid level (Charve & Reineccius, 2009). However, increasing the solid level too much may result in non dissolved material and increased viscosity, which may slow down the film formation. Therefore there must be optimum solid concentration. High temperature exposure results in the build-up of a more porous crust that offers less resistance to carvacrol vapour diffusion during drying. The retention of aroma compounds is dependent on its diffusivity after the formation of a semi permeable surface. Diffusion and volatilization pathways are shorter; consequently more carvacrol is lost (Fig. 5 and Table 3).

3.4.3. *Influence of additives*

Drying time and thicknesses are mainly dependent on CS and glycerol, whereas retention is directly glycerol and NC content dependent. An increased concentration of additives led to a greater molecular contact between the chitosan and the compounds. Therefore, the polymer chain forces were weakened and the matrix network more opened. Chitosan films of high deacetylation degree that have higher crystallinity are more mobile (Chen, Lin, & Yang, 1994). Increased macromolecular chain mobility facilitated carvacrol uptake (from 1.35 to 4.94 and from 9.46 to 18.90 for chitosan 1 and 2% (w/v) respectively). Glycerol displaced bound water and acetic acid. It resulted in an increased number of existing bonds and in the formation of new bonds (with chitosan —NH groups)

Table 3
Composition of the chitosan based films and its influence on the thickness, drying time, carvacrol retention and the air/biopolymer partition coefficient when dried at 20 °C and 30% RH.

Film	Chitosan dry matter (g)	Glycerol % (w/p.d.m.)	Nanoclays or arabic gum % (w/p.d.m.)	Thickness (μm)	Drying time (h)	Carvacrol retention (%)	$K_{mass} \times 10^{-6}$
Chitosan film	1	0	0	28 ± 3	14	1.35 ± 0.06^{g}	$6.13 \pm 0.49^{b,c,d}$
	2	0	0	50 ± 5	14	9.45 ± 0.15^{c}	3.88 ± 0.19^{e}
	3	0	0	58 ± 5	18	$4.24 \pm 0.10^{d,e}$	$5.53 \pm 0.65^{c,d,e}$
Chitosan film with glycerol	1	30	0	35 ± 3	14	4.96 ± 0.14^{d}	0.85 ± 0.18^{f}
	2	30	0	55 ± 5	14	18.90 ± 1.51^{b}	0.52 ± 0.08^{f}
	3	30	0	60 ± 5	18	$1.83 \pm 0.31^{f,g}$	$7.70 \pm 0.13^{a,b}$
Chitosan film with nanoclays	1	0	10	na	12	NA	NA
	2	0	10	78 ± 6	12	$2.42 \pm 0.37^{f,g}$	$5.71 \pm 0.68^{c,d,e}$
	3	0	10	na	13	NA	NA
Chitosan film with glycerol and nanoclays	1	30	10	95 ± 10	14	28.17 ± 0.89^{a}	0.47 ± 0.08^{f}
	2	30	10	110 ± 10	14	$21.31 \pm 0.22^{a,b}$	0.59 ± 0.01^{f}
	3A	30	10	110 ± 10	16	8.49 ± 0.03^{c}	1.68 ± 0.30^{f}
	3B	30	10	110 ± 10	16	9.23 ± 0.93^{c}	1.40 ± 0.78^{f}
Chitosan/arabic gum blends with glycerol	3	30	3	95 ± 10	12	$4.63 \pm 0.09^{d,e}$	$4.98 \pm 0.98^{d,e}$
	3	40	3	118 ± 11	12	$3.22 \pm 0.14^{e,f}$	$7.14 \pm 0.81^{a,b}$
	3	50	3	138 ± 13	13	$1.78 \pm 0.15^{f,g}$	8.48 ± 1.92^{a}

NA, not measured because produced films were too brittle. Different superscripts (a–g) within a column indicate significant differences among formulations ($p < 0.05$).

(Cerqueira, Souza, Teixeira, & Vicente, 2012; Park, Jun, & Marsh, 2001). In this sense, glycerol was a competitive agent for carvacrol, as it occupied places where aroma compound could interact.

At higher concentrations (40 and 50% of polymer dry matter) glycerol facilitated the mobility of polymer chains, disturbed gelation and film formation. Thus, drying time was longer and more carvacrol was lost at all temperature ranges. Likely, it changed the film thickness and enhanced the carvacrol diffusion into the atmosphere (Table 3). Similar findings were reported by Sebti, Chollet, Degrave, Noel, and Peyrol (2007). Sánchez-González et al. (2011) observed a reduction in the film thickness by means of essential oil evaporation. To sum up, both unplasticized and plasticized films had lower thickness as polymer dry matter decreased. It seems that temperature had no significant effect on these film formulations (Fig. 6).

When nanoclays were mixed in FFS, only films with glycerol could be obtained. Lavorgna, Pischelli, Mangiacapra, and Buonocuore (2010) stated that in films without glycerol, NC stacks lay with their platelet surface parallel to the casting surface. Brittleness is due to the complex/branched primary structure and weak intermolecular forces of natural polymers (Günister, Pestreli, Ünlü, Atici, & Güngör, 2007). These authors indicated interlayer distances of 16.34 Å, thereafter carvacrol (size 6.62–10.14 Å) could easily enter the free spaces of unfilled gaps. Perisco et al. (2009) reported that carvacrol is able to enter the clay galleries enhancing the lamellar distance up to 38.4 Å. Statistical analyses did not show significant effects between A and B processing sequences of CS/NC films. NC stacks were randomly orientated in the space. In a previous study, Wang et al. (2005) reported that the size of some stacks reached even up to 600 nm. That might be the reason why NC aggregates appeared in the tested samples. Mascheroni, Chalier, Gontard, and Gastaldi (2010) suggested that increased carvacrol retention capacity exhibited by NC/wheat gluten was due to the entrapment of the carvacrol molecules in the aggregated structures due to specific interactions between the different components of the mixture. The interaction between solvents and clays controls the dispersion of the platelets and, as a consequence, it determines the resulting properties of nanocomposites (Burgentzlè, Duchet, Gerard, Jupin, & Fillon, 2004). Glycerol enhanced the intercalation of chitosan in the silicate galleries and hindered flocculation. Moreover it reduced the surface energy of the aqueous solution and it extended the H-bonding between CS–NC (Shanmugharaj, Rhee, & Ryu, 2006). Therefore, enough gaps and better carvacrol dispersion were achieved (Darder, Colilla, & Ruiz-Hitzky, 2003). As a consequence, retention increased 21, 2.6 and 2.2 times in films containing 1, 2 and 3% (w/v) chitosan, respectively. Even though the volatility and consequently the carvacrol loss from CS matrix were higher at higher temperatures, the addition of NC seemed to protect the carvacrol molecule. There are two possible explanations – a reduced drying time (at 100 and 60 °C drying time decreases 3 times) and the thermal stability of formed structures. Indeed, the authors pointed out that NC/carvacrol decomposed at higher temperatures (Keawchaoon & Yoksqn, 2011). Carvacrol losses from soy protein isolate coated papers were up to 37% when processed at 90 °C, being higher than those prepared at 25 and 50 °C (12–14%, respectively).

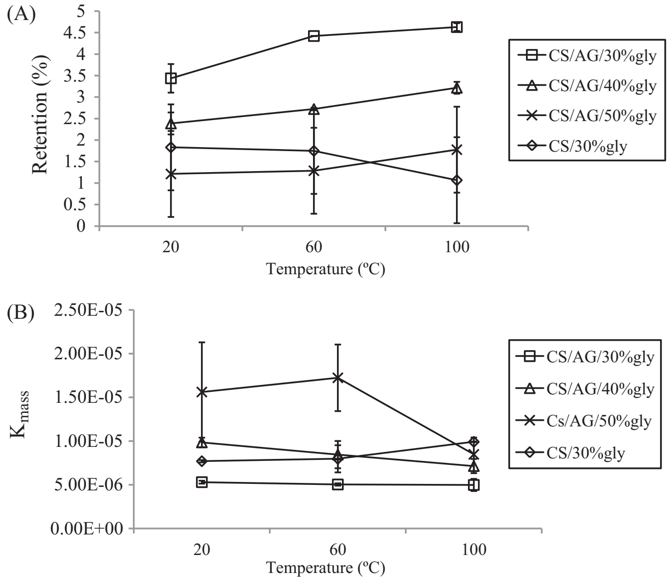

Fig. 6. Retention and partition coefficient (K_{mass}) of carvacrol as a function of the film composition and the drying temperature. CS – chitosan, AG – arabic gum, gly – glycerol.

Arabic gum and chitosan mainly form coacervates. They are liquid-like, mobile and reversible structures formed as a result of low charge density in systems where the ionic interaction of opposite polyelectrolyte is of moderate strength (Biesheuvel & Cohen Stuart, 2004). The formation of soluble complexes is attributed to a chitosan lower charge density and the presence of non-charged monomers (Coelho et al., 2011). Therefore during the homogenization of carvacrol molecules they can enter the coacervate CS/AG globules which protected the aroma compound from evaporation. Differences in the nature of biopolymers increased the formation of a bubbled structure (Alcantara, Rumsey, & Krochta, 1998). This was not the case for pure chitosan films. In the presence of AG, the retention rate increased from 2.5 up to 3.2 folds, in a range from 20 to 100 °C, respectively. Whereas the polymer dry matter increased by 50%, the incorporation of AG shortened the drying time 19 and 32 times in the interval from 20 to 100 °C, respectively. Better film forming and emulsifying properties of AG increased essential oil retention from 45 to 63% with an AG concentration increase (Fernandes, Oliveira, Sztatisz, & Novák, 2008; Krishnan, Kshirsagar, & Singhal, 2005).

4. Conclusions

This paper demonstrates the importance of controlling both composition and process parameters during the drying of biopolymer active films. In relation to the effectiveness of potential antimicrobial packaging, it is very important to know the amount of active compound retained in the film matrix after processing. Moreover, the film structure, including additives, influenced the aroma compound retention and the release from the films. An appropriate solvent that influenced FFS also affected film drying (kinetic and time) and therefore the structure which governs the film functional properties (aroma compound retention, release and film appereance). Drying time was reduced when hydroalcoholic solutions were used for polymer powder dissolution. The partition coefficient can be related to carvacrol retention during film processing (casting plus drying) in chitosan based films. The glycerol-plasticized samples displayed higher water content due to the high hydrophilic character of glycerol. The loss of aroma compound was greatly influenced by the water content in the drying film. Drying curves did not display linear trend. It was due to the structural changes in the thin layer of FFS because the diffusion was modified according to the time. It seemed that the drying time and thickness were mainly dependent on the CS and glycerol content, whereas the retention was directly correlated to the glycerol and NC content. Disrupting intermolecular forces between polymer chains, plasticizers changed the matrix structure that facilitated the carvacrol uptake. Moreover the addition of AG favoured carvacrol retention. Retention rate increased up to 3 folds in temperatures ranging from 20 to 100 °C. The presence of nanoclays in plasticized films promoted the aroma compound retention which was more pronounced at lower drying temperatures. Further investigation will be focused on the effect of the partition coefficient on the antimicrobial and/or antioxidant activities of such bioactive films.

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