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Effect of Composition, Stability and Microstructure of O/W Emulsions on the Retention and Release Characteristics of Diacetyl and (-)-Alpha-Pinene

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The influence of natural emulsifiers or their mixtures with synthetic emulsifiers (surfactants) on the retention, release characteristics and odor intensity of hydrophilic diacetyl and hydrophobic (-)-α-pinene in oil-in-water emulsions was examined. A lipid fraction was composed of rapeseed oil, whereas sodium caseinate, concentrate of soy proteins, dried egg yolk, sodium dodecyl sulfate (SDS), polyoxyethylene sorbitan monostearate (Tween 60) and polyoxyethylene sorbitan tristearate (Tween 65) were used as emulsifiers. The hydrophilic-lipophilic balance (HLB) of surfactants was within the range of 11–40. The thermodynamic study showed that natural emulsifiers and surfactants affected more retention of hydrophobic aroma compound than the one of hydrophilic odorant. Release rate constants of (-)-α-pinene were observed to decrease mainly with lowering the HLB value of surfactants or their mixtures, whereas the ones of diacetyl with increasing viscosity of the system. Microstructural properties of the emulsions *i.e.* surface area of the oil-water interface, surface protein concentration and the dispersion index, had no statistically significant effect on the release rate of hydrophobic aroma compound. Odour intensity detected orthonasally was higher correlated with headspace concentration of aroma compounds, measured by gas chromatography-mass spectrometry, in relation to (-)-α-pinene than diacetyl.

INTRODUCTION

Emulsions are categorized in terms of the relative location of the oil and water phases within the system. A system that consists of oil particles dispersed in an aqueous phase is referred to as an oil-in-water (o/w) emulsion [van Ruth *et al*., 2002b; Capek, 2004]. In the food industry o/w emulsions are mainly typified by milk, mayonnaises and salad dressings [Dalgleish, 1997; Charles *et al*., 2000; Mandala *et al*., 2004]. Moreover, such systems can be also used as ingredients, which participate in the formation of the structures of more complex products. Of such types are yoghurts or other gelled systems containing emulsion droplets that must interact with other food ingredients, but not be destabilized in the process [Dalgleish, 2006; Nongonierma *et al*., 2006]. Oil-in-water emulsions exhibit all classical behaviors of metastable colloids and their kinetic and thermodynamic stability greatly depends on the adsorbed layer at the oil-water interface [Dickinson, 1997; Hemar & Horne, 1998; Capek, 2004]. Emulsifiers used in the food industry may be roughly divided into small amphiphilic molecules – surfactants *e.g.* polysorbates, monoacylglycerols, diacylglycerols and lecithins, with molecular masses in the range of 500–1300 Da and large surface-active molecules, such as proteins, which have molecular masses of tens of kilodaltons [Dalgleish, 1997; Stauffer, 2001]. Depending on their nature, the amphiphilic molecules adsorbed at the interface, exhibit different stabilization mechanisms [Cornec *et al*., 1996; Kong *et al*., 2001]. Proteins form a hydrodynamically thick layer around the surface of the oil droplets and being generally charged can stabilize emulsion droplets by both steric and electrostatic mechanisms [Phillips, 1981; Dalgleish, 2006]. In contrast, surfactant molecules do not form a viscoelastic surface, they are more surface-active than proteins and form a compact adsorbed layer. This layer relies on charge repulsion or the Gibbs-Marangoni mechanism [Cornec *et al*., 1996; Wilde *et al*., 2004]. Emulsions stabilized by mixtures consisting of proteins and surfactants demonstrate all the above-discussed types of stabilization [Kong *et al*., 2001]. It has been reported that small molecule surfactants have important effects on the composition of the interfacial layer. In general terms, most surfactants competitively displace proteins from the interface [Dickinson & Ritzoulis, 2000; Bortnowska, 2008]. The degree of displacement depends not only on the specific surfactant used, but also when it is added *i.e.* before or after emulsion formation. If the surfactant is introduced before emulsification, partial displacement is found, and if the surfactant is added after emulsification, complete displacement may occur [Euston *et al*., 1995; Fang & Dalgleish, 1996]. However, evidence also exists for mixed surfactant-protein structures, adsorbed at the interface or co-operative adsorption [Lu & Lundahl, 1996; Dickinson & Rit-

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zoulis, 2000; Demetriades & McClements, 2000; Malhotra & Coupland, 2004; Dalgleish, 2006].

Release and retention of aroma compounds in o/w emulsions is mainly dependent on the affinity of volatile compounds to the liquid phases and their interactions with non-volatile ingredients such as proteins and carbohydrates [Fisher & Widder, 1997; Druaux & Voilley, 1997; Charles *et al*., 2000; Reineccius, 2006]. In general, release of aroma compounds from the oil phase proceeds at a lower rate than from the aqueous phase. This has to be attributed to the higher resistance to mass transfer in oil than in water and to the fact that in o/w emulsions hydrophobic odorants, found in majority in food products, have first to be released from oil to aqueous phase before they can be released from the aqueous phase to the headspace [Leland, 1997; de Roos, 1997; Seuvre *et al*., 2000]. Moreover, release of aroma compounds from o/w emulsions can be affected by microstructure characterized by the surface area of the lipid-water interface and the nature and amount of the surface-active agents adsorbed at the interface as well as by texture *i.g.* viscosity, emulsion droplet size, *etc*. Furthermore, the rate of surface renewal and the surface area of the product should also be considered [de Roos, 1997; Harrison *et al*., 1997; Charles *et al*., 2000]. The general information regarding the influence of the food matrix on the volatility of aroma compounds under equilibrium conditions can be provided by partition coefficient, defined as the ratio of the concentration of the aroma compound in the gas phase to its concentration in the liquid phase [Landy *et al*., 1996; van Ruth *et al*., 2000; Reineccius, 2006]. The partition coefficient can be measured either by static headspace methods at equilibrium or by dynamic headspace methods. Among the static methods, direct and indirect measurements can be distinguished [Kolb & Ettre, 1997; Savary *et al*., 2006]. A large number of studies suggest that the presence of proteins and surfactants, depending on their ratio and concentration, may affect microstructure, stability and texture of o/w emulsions however, there are no reports showing how the extent of these changes may affect the retention and release rate of flavour compounds in relation to their hydrophobicity.

The aim of this research was to determine the impact of different matrices (o/w emulsions) stabilized by both natural emulsifiers and surfactants on the retention and release characteristics of diacetyl and (-)-α-pinene.

MATERIALS AND METHODS

Materials

Aroma compounds: diacetyl and (-)-α-pinene with purities higher than 98% were bought from Sigma-Aldrich Sp. z o.o. (Poznań), their physicochemical characteristics and odor description are given in Table 1. Natural emulsifiers: spray-dried sodium caseinate, concentrate of soy proteins (Danpro S-760) and dried egg yolk were bought from: Duncean (Kamień Pomorski), Central Soya (Warszawa) and Hortimex (Konin), respectively. Synthetic emulsifiers (surfactants): sodium dodecyl sulfate (SDS), polyoxyethylene sorbitan monostearate (Tween 60) and polyoxyethylene sorbitan tristearate (Tween 65) were purchased from Sigma-Aldrich (Poznań). Values of their hydrophilic-lipophilic balance (HLB) are shown in Table 2. Commercial rapeseed oil "Kujawski" (ZT "Kruszwica" S.A.) was bought from a local supermarket and used without further treatment. According to manufacturer's specification, its triglyceride composition was as follows: 7 wt% saturated, 65 wt% monounsaturated and 28 wt% polyunsaturated fatty acids. The oil did not contain any antioxidant. Distilled water was used in all solutions. All chemicals applied for analyses were of analytical grade and purchased from Hartim (Szczecin).

TABLE 1. Physicochemical characteristics of diacetyl and (-)-α-pinene.

Aroma compound	Molecular weight (g/mol)	Molar volume[a] (cm^3/mol)	Hydrophobicity[b] Log *P*	Density[b] (g/mL)	Odor descriptor[c]
Diacetyl	86.09	96.2	-0.47	0.985	buttery
(-)-α-Pinene	136.24	207.2	4.83	0.858	pine-like

[a]Calculated according to Całus [1987]; [b]Sigma-Aldrich (Poznań) at 20°C; [c]van Ruth *et al*. [2002a].

TABLE 2. Codes, composition (wt %) of the emulsions[a] and HLB values of surfactants and their mixtures.

Code	Sodium caseinate	Code	Danpro S-760	Code	Dried egg yolk	Oil	Buffer	SDS	Tween 60	Tween 65	HLB
A1	2	B1	2	C1	2	20	78	–	–	–	–
A2	1	B2	1	C2	1	20	79	–	–	–	–
A3	1	B3	1	C3	1	20	78	1	–	–	40
A4	1	B4	1	C4	1	20	78	0.7	–	0.3	31
A5	1	B5	1	C5	1	20	78	0.4	–	0.6	23
A6	1	B6	1	C6	1	20	78	–	1	–	15
A7	1	B7	1	C7	1	20	78	–	–	1	11

[a]Emulsions: A3-A7, B3-B7 and C3-C7 are characterized by the same composition and concentration of surfactants.

Emulsion composition and preparation

Twenty-one oil-in-water emulsions were prepared. Their composition and the codes further used in the analyses are shown in Table 2. The oil content was kept at a constant level of 20 wt%. The emulsions denoted: A, B and C were prepared with surfactants and/or natural emulsifiers: sodium caseinate, concentrate of soy proteins and dried egg yolk, respectively. The matrix effect of the hydrophilic-lipophilic balance (HLB) on microstructural properties of emulsions as well as retention and release characteristics of aroma compounds was studied by the addition of surfactants or their mixtures with HLB within the range of 11–40 (Table 2). The HLB values of mixtures consisting of surfactants were calculated according to Stauffer [2001]. Aqueous phases of emulsions were prepared by dissolving appropriate amount of surface-active materials in buffer (disodium hydrogen phosphate-citric acid) at pH 7 with 0.02 wt% sodium azide added to protect against microbial contamination. The o/w emulsions were produced by homogenizing rapeseed oil and aqueous solution together for 30 s at 14,000 rpm using an MPW 302 laboratory homogenizer (Med. Instruments, Warszawa). After preparation, the emulsions were degassed under vacuum in APT Line Serie VD desiccator (Binder GmbH, Germany) for 1 h to remove all gas bubbles produced during mixing. For the chromatographic and sensory analyses, a precise amount of diacetyl or (-)-α-pinene was added to the emulsions, to give a final concentration of 0.1 wt%. Then the samples were stirred for 30 s at 1200 rpm to ensure equal distribution of the aroma compounds, and the jars were hermetically sealed.

Particle size determination

Sample droplet size and distribution were measured using a Matic®B1 microscope equipped with a built in camera (Carlzeiss Jena, Germany). Objective lens calibrated with an objective micrometer and an appropriate software (Multiscan v. 11. 06, Computer Scanning Systems) were used. Aliquots of fresh samples were observed after 1:20 dilution with distilled water. Over 400 oil droplets were measured to estimate the average droplet size using 6 fields per test [Quintana *et al*., 2002]. The size distribution of the emulsion droplets was expressed by arithmetical average droplet diameter – D (μm) and polydispersity – P, calculated as the variance of the log-normal distribution function [Chung *et al*., 2001]. The volume-surface mean diameter – D[3,2] (μm) and the volume-weighted mean diameter – D[4,4] (μm) were calculated from the equations: $D[3,2] = \sum n_i d_i^3 / \sum n_i d_i^2$ and $D[4,3] = \sum n_i d_i^4 / \sum n_i d_i^3$, where: n_i - number of droplets of diameter d_i [Dickinson & James, 1999; Dickinson & Merino, 2002]. Dispersion index – DI, being a measure of the width of the size distribution was calculated as follows: $DI = (D_{v,0.9} - D_{v,0.1})/D_{v,0.5}$, where: $D_{v,0.9}$ – diameter under which 90% of the oil droplets in the cumulative distribution fall, $D_{v,0.1}$ – diameter corresponding to 10% of oil droplets and $D_{v,0.5}$ – diameter corresponding to 50% of the droplets – median droplet diameter [Joscylene & Trägärdh, 1999; Charles *et al*., 2000].

Assessment of emulsion stability

Stability of the emulsions was assessed towards creaming using accelerated ageing [Huang *et al*., 2001] and in terms of changes in the average particle size parameter expressed by volume-weighted mean diameter – D[4,3] (μm). The D[4,3] parameter was related to the thermodynamic stability of the emulsions and calculated as explained above [Dickinson & James, 1999; Capek, 2004]. For the measurements of creaming stability, 8±0.1 mL of each emulsion were placed into 10 mL plastic tubes and centrifuged using an MPW-350 laboratory centrifuge (Med. Instruments, Warszawa) at 1983.6×*g* for 10 min at a room temperature (22±0.5°C). Creaming stability of the emulsions – ES (%), was calculated as a percentage: ES (%) = (remaining emulsion height/initial emulsion height) × 100.

Surface protein concentration

The surface protein concentration Γ (mg/m^2) was calculated from the difference in the amount of protein used to prepare the emulsion and that measured in the subnatants after centrifugation (at 1983.6×*g* for 10 min at 22±0.5°C) divided by the total emulsion surface (S_T). The S_T (m^2) was derived from the equation: S_T = (mL oil) × SSA, where: specific surface area – SSA (m^2/mL oil) was calculated as follows: SSA = 6/D[3,2] [Cornec *et al*., 1996; Diftis & Kiosseoglou, 2004; Soottitantawat *et al*., 2005]. The nitrogen content in the initial aqueous phase and in aqueous layer of the emulsion was determined by the Kjeldahl procedure with a Tecator Kjeltec system (Tecator AB, Höganäs, Sweden) [AOAC, 1995]. The factors of: 6.68, 6.38 and 6.25 for: dried egg yolk, sodium caseinate and concentrate of soy proteins, respectively were used to convert nitrogen to protein content [AOAC, 1995].

Viscosity

Apparent viscosity of the emulsions was measured at a room temperature (22±0.5°C) using a viscometer Rheotest 2–50 Hz – type RV 2 (Medingen, Germany) equipped with S/S1 cylinder, at a shear rate of 1312 s^{-1}.

Static headspace analysis

Gas chromatography-mass spectrometry (GC-MS) analyses were performed on a Perkin-Elmer AutoSystem XL GC apparatus coupled with TurboMass MSD (Mass Selective Detector Quadrupole type). For the analyses of diacetyl and (-)-α-pinene relative retention, 5±0.01 mL of each emulsion were transferred into 22.3 mL headspace vials and the vials were immediately sealed. Then the emulsions were incubated in an ST1 thermostat (Pol-Eko-Aparatura, Wodzisław Śl.) at 37±0.5°C until thermodynamic equilibrium was reached. Preliminary experiments at different equilibrium times were conducted to ensure that the analysis for each sample was performed at equilibrium. The time periods of 3 h and 24 h were sufficient to reach equilibrium in each emulsion for diacetyl and (-)-α-pinene, respectively. The parameters of the release rate of the investigated aroma compounds were determined with following procedures. Open headspace vials containing 5±0.01 mL of each emulsion were stored in a refrigerator at 4±0.5°C for 1–10 days and at fixed time intervals the samples were removed from the refrigerator in order to measure the residual amount of diacetyl or (-)-α-pinene. For the chromatographic analyses, headspace vials containing emulsions were placed into a Perkin-Elmer

TurboMatrix 16 autosampler and after equilibration 1 mL of headspace sample was automatically withdrawn and injected into a gas chromatograph. Only one sample per headspace vial was made. The inlet was operated in the splitless mode and the carrier gas was helium, at a flow rate of 0.7 mL/min. Separation was done using a PE-5MS capillary column (30 m × 0.25 mm × 0.25 μm) by increasing oven temperature from 50 to 150°C at a rate of 10°C/min. The reference solutions were analysed daily. The headspace components were identified by comparison of their mass spectra with those of authentic samples or with data from the literature. Quantitative analyses were performed using an external standard method as described by Kolb & Ettre [1997].

Relative retention and release rate characteristics calculations

Relative retention of diacetyl and (-)-α-pinene in the emulsions was defined as the percentage of their volatility decrease relatively to buffer at the same temperature and calculated from the equation: $R_{relative}$ (%) = (1 – K_{matrix} /K_{buffer}) × 100, where: K_{matrix} – vapour-matrix partition coefficient and K_{buffer} – vapour-buffer partition coefficient (expressed in mass fractions). The results were interpreted that the higher the volatility decrease in the matrix, the higher the retention of the aroma compound [Nongonierma *et al*., 2007]. Partition coefficients: K_{matrix} and K_{buffer} were calculated according to methods described by Kolb & Ettre [1997] and Seuvre *et al*. [2006]. The parameters of the release rate of the studied flavour compounds were calculated by Avrami's equation: R = exp[– (kt)^n], where: R – retention defined as the ratio of the remaining amount of (-)-α-pinene or diacetyl in the emulsion to the initial one, t – storage time, k – release rate constant, n – parameter representing release mechanism [Soottitantawat *et al*., 2005].

Sensory analysis

Odor intensity was evaluated by the method using a scale. The internal panel consisted of 12 assessors (8 women and 4 men), 20–24 years old, selected for their capacity to recognize and memorize odors according to Polish Standard [PN-ISO 8586–1:1996]. During 6 sessions of 45 min each over a period of 2 weeks, the assessors were trained to evaluate the smell of diacetyl and (-)-α-pinene added in selected samples and finally they were familiarized with the 10-point scale [PN-ISO 5496:1997]. The reference samples contained buffer and were flavored with diacetyl or (-)-α-pinene at: 0.00, 0.025 0.05, 0.075 and 0.10 wt% and the appropriate odor intensity was quantified as: 0, 2.5, 5, 7.5 and 10 points or was calculated using linear interpolation. The incubation of the emulsions was done using the same procedures as for the measurements of relative retention of the studied odorants. Samples were served in individual randomized order at a room temperature (22±0.5°C) in 50 mL jars with metal lids, marked by a three-digit code. All testing sessions were limited to the emulsions formed with one natural emulsifier and its mixtures with surfactants, separately for each examined odorant. Panelists were instructed to smell each sample and rate its intensity. Between each rating, the assessors eliminated any odor remaining in the nose by sniffing a pure water sample.

Statistical analysis

Three replicates were conducted for all measured parameters and data were statistically treated using STATISTICA for Windows (version 6.0, Copyright© Statsoft, Inc. 2003). A two-way analysis of variance (ANOVA) was carried out on the vapour-matrix partition coefficients (K_{matrix}) of diacetyl or (-)-α-pinene, found in emulsions prepared with natural emulsifiers and surfactants as variables. Significant differences between means were identified by the least significant difference (LSD) procedure, using Tukey's multiple comparison test (α≤0.05). The extent of correlation between investigated parameters was determined by Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Characteristics of the emulsions

The microstructural properties of the emulsions are displayed in Table 3. An increase of natural emulsifier concentration from 1 to 2 wt% caused a significant (α≤0.05) change in arithmetical average droplet diameter (D) from 0.39 μm (P=0.092) to 0.32 μm (P=0.078), in emulsions prepared with a concentrate of soy proteins (B2-B1). On the other hand, the lowest D value of 0.28 μm was registered in emulsions

TABLE 3. Microstructural properties of emulsions: arithmetical average droplet diameter (D), polydispersity (P), dispersion index (DI), median droplet diameter ($D_{v,0.5}$), specific surface area (SSA) and surface protein concentration (Γ).

Code	D (μm)	P	DI	$D_{v,0.5}$ (μm)	SSA (m^2/mL)	Γ (mg/m^2)
A1	0.28	0.046	2.06	0.19	9.52	2.57
A2	0.28	0.044	2.47	0.19	9.84	1.33
A3	0.27	0.026	2.05	0.19	12.77	1.54
A4	0.26	0.021	1.95	0.19	14.63	0.93
A5	0.23	0.019	2.21	0.17	15.01	1.28
A6	0.19	0.015	2.82	0.10	17.14	0.76
A7	0.16	0.013	3.36	0.07	17.65	0.75
$LSD_{0.05}$	0.04	–	–	–	1.23	0.19
B1	0.32	0.078	3.75	0.17	7.89	1.93
B2	0.39	0.092	4.58	0.18	6.19	1.07
B3	0.24	0.049	3.11	0.14	8.96	0.69
B4	0.23	0.038	2.53	0.14	9.84	0.39
B5	0.27	0.044	2.38	0.17	9.84	0.51
B6	0.24	0.037	2.53	0.15	9.84	0.59
B7	0.21	0.041	4.29	0.10	9.52	0.41
$LSD_{0.05}$	0.06	–	–	–	0.92	0.14
C1	0.28	0.055	2.78	0.17	8.57	1.38
C2	0.30	0.048	2.40	0.20	9.84	0.91
C3	0.26	0.051	3.87	0.15	9.09	0.36
C4	0.24	0.028	2.34	0.16	11.76	0.32
C5	0.25	0.042	3.31	0.14	9.84	0.61
C6	0.25	0.039	3.27	0.15	10.53	0.44
C7	0.24	0.058	3.18	0.15	6.89	0.51
$LSD_{0.05}$	0.05	–	–	–	1.08	0.12

formed with sodium caseinate (A1, A2) and dried egg yolk (C1). Median droplet diameter ($D_{v,0.5}$) did not change in sodium caseinate-stabilized emulsions (A1, A2), whereas in those prepared with soy proteins (B1, B2) or dried egg yolk (C1, C2), this parameter decreased only imperceptibly (Table 3). The lack of changes in particle diameter in emulsions containing 1 and 2 wt% sodium caseinate (A1, A2) could be associated with excellent emulsifying properties of caseins, and 1 wt% of this emulsifier was probably sufficient to lower interfacial tension, form resistance to coalescence and produce relatively low oil droplets [Philips, 1981; Dickinson, 1999; Dalgleish, 2006]. A decrease in particle diameter with a higher concentration of soy proteins (B2-B1) may be related to their physicochemical properties. Soy proteins are poorly soluble and an increase of this emulsifier concentration may have improved its emulsifying activity [Malhotra & Coupland, 2004]. A small decrease in particle diameter observed between emulsions C2-C1 was likely to be connected with protein concentration. Egg yolk contains less proteins than sodium caseinate, whereas other surface-active constituents present in this emulsifier, however exhibiting strong propensity to adsorb at the oil-water interface, were probably less effective than proteins [Aluko & Mine, 1998; Anton *et al*., 2000]. Considering emulsions formed with 2 wt% emulsifiers (natural or mixtures with surfactants), the highest significant ($\alpha \leq 0.05$) difference regarding diameter D of 0.12 μm was observed between emulsions A1-A7 whereas the lowest one of 0.04 μm between samples C1-C7. The smallest D value of 0.16 μm ($P=0.013$) was recorded in emulsion formed with 1 wt% sodium caseinate and 1 wt% Tween 65 (A7), whereas the highest value of this parameter of 0.32 μm was observed in the sample prepared with a 2 wt% concentrate of soy proteins (B1) (Table 3). The median diameter was within ranges of: 0.19–0.07 μm, 0.17–0.10 μm and 0.17–0.15 μm in the case of samples: A1-A7, B1-B7 and C1-C7, respectively. There was no clear dependence between HLB and dispersion index (DI) of the emulsions (A3-A7, B3-B7 and C3-C7). The lowest DI value of 1.95 was found in sample A4, whereas the highest one of 4.29 in emulsion B7. The generally observed trend to decrease droplet diameter with lowering HLB value is not consistent with data reported by Quintana *et al*. [2002] who suggested the opposite effect. However, they used only surfactants to prepare emulsions and mixtures of natural and synthetic emulsifiers applied in this experiment could have behaved otherwise. In the absence of surfactants surface protein concentration (Γ) increased with concentration of natural emulsifiers increasing from 1 to 2 wt%. The highest significant ($\alpha \leq 0.05$) difference in the amount of adsorbed proteins of 1.24 mg/m^2 was observed between sodium caseinate-stabilized emulsions (A2-A1), whereas the lowest one of 0.47 mg/m^2 ($\alpha \leq 0.05$) between samples C2-C1, formed with dried egg yolk (Table 3). These results suggest that the quantity of adsorbed proteins was dependent on their initial concentration in the aqueous phase and are in agreement with findings reported by Euston *et al*. [1995] and Bortnowska [2009]. However, the differences in adsorption of proteins, observed between emulsions containing various natural emulsifiers could also be associated with differentiated surface hydrophobicity of proteins [Phillips, 1981; Diftis & Kiosseoglou, 2004]. The presence of surfactants or their mixtures with a lowering HLB value generally decreased surface protein concentration in emulsions prepared with 1 wt% natural emulsifier (Table 3). The highest significant ($\alpha \leq 0.05$) difference of 0.66 mg/m^2 was identified between samples B2-B7, whereas the lowest one of 0.40 mg/m^2 ($\alpha \leq 0.05$) between emulsions C2-C7 (Table 3). The microstructural changes of emulsions formed with mixtures consisting of proteins and surfactants are consistent with those reported by Dickinson & James [1999], Demetriades & McClements [2000] and Bortnowska [2008] and indicate that surface protein concentration was mainly affected by competitive adsorption between surface-active components. However, not linear relationship between HLB and the amount of adsorbed proteins may also suggest that the nature of the surfactant-protein interactions at the interface and in the bulk continuous phase could have influenced this phenomenon [Demetriades & McClements, 2000; Diftis & Kiosseoglou, 2004]. Moreover, replacement of proteins by surfactants was also likely to affect droplet diameter due to their ability to rapidly lower interfacial tension [Euston *et al*., 1995; Capek, 2004; Dalgleish, 2006].

Stability of the emulsions

The results of thermodynamic stability expressed by volume-weighted mean diameter (D[4,3]) and stability towards creaming of the emulsions are presented in Table 4. Considering emulsions prepared with natural emulsifiers, an increase in concentration from 1 to 2 wt% inconsiderably increased stability towards creaming in emulsions formed with

TABLE 4. The volume-weighted mean diameter (D[4,3]) and stability towards creaming (ES).

Code	D[4,3] (μm)	ES (%)	Code	D[4,3] (μm)	ES (%)	Code	D[4,3] (μm)	ES (%)
A1	0.76	25.23	B1	0.87	27.68	C1	0.84	28.42
A2	0.74	24.16	B2	1.21	26.39	C2	0.72	30.48
A3	0.56	28.19	B3	0.83	27.61	C3	0.79	27.91
A4	0.48	25.39	B4	0.77	26.72	C4	0.67	27.52
A5	0.48	25.48	B5	0.74	27.19	C5	0.73	25.64
A6	0.43	29.01	B6	0.78	27.17	C6	0.70	30.43
A7	0.44	33.47	B7	0.78	28.83	C7	1.27	25.44
$LSD_{0.05}$	0.04	1.18	$LSD_{0.05}$	0.05	1.22	$LSD_{0.05}$	0.11	1.46

sodium caseinate (A2-A1), whereas significantly (α≤0.05) in those stabilized by soy proteins (B2-B1). In contrary, a significant (α≤0.05) decrease in stability from 30.48 to 28.42% was observed between emulsions C2-C1, prepared with dried egg yolk (Table 4). Regarding thermodynamic stability, the change of natural emulsifier concentration from 1–2 wt%, decreased significantly (α≤0.05) D[4,3] parameter of 0.34 μm in the soy proteins-stabilized emulsions (B2-B1) and increased it from 0.72 to 0.84 μm in the emulsions formed with dried egg yolk (C2-C1). The increase of the stability measured towards creaming along with an increasing concentration of natural emulsifiers used in this experiment, may be attributed to the fact that proteins provide not only steric and electrostatic stabilization but also exhibit water-holding capacity and gel formation and these properties could have contributed to the observed stability [Phillips, 1981; Khwaldia *et al*., 2004; Dalgleish, 2006]. Moreover, the relatively high values of creaming stability found in the samples containing 1 and 2 wt% dried egg yolk may be related to excellent emulsifying properties of lipoproteins whose amphipathic character probably allowed them strongly interact with the oil-water interface [Aluko & Mine, 1998; Anton *et al*., 2000; Bortnowska & Tokarczyk, 2009]. Furthermore, the presence of phospholipids, such as lecithins, may have also improved stability in the samples containing egg yolk due to their ability to adsorb together with proteins [Dalgleish, 2006]. Addition of surfactants with a lowering HLB value to the emulsions prepared with 1 wt% natural emulsifier, increased their stability measured towards creaming and these changes were found significant (α≤0.05) between majority of the samples (Table 4). The highest increase in stability of 9.31% (α≤0.05) was observed between emulsions A2-A7, whereas in the samples formed with dried egg yolk (C2-C7), the addition of surfactants caused a decrease in stability from 30.48 to 25.44%. The lowering HLB value of surfactants was observed to significantly (α≤0.05) decrease D[4,3] diameter from 0.74 to 0.44 μm in emulsions A2-A7 and from 1.21 to 0.78 μm in samples B2-B7. In contrast, in the emulsions made with dried egg yolk (C2-C7), the value of this parameter increased from 0.72 to 1.27 μm (Table 4). Improvement of creaming stability in emulsions formed with sodium caseinate and SDS or Tween 60 was reported by Bortnowska [2009]. In the case of sodium caseinate-SDS interactions Dickinson & Ritzoulis [2000] suggested that this phenomenon might be explained in terms of a considerable amount of SDS binding to the protein, which reduced the amount of SDS available to promote protein displacement and depletion flocculation. It seems that positive proteins-surfactants interactions probably led to improvement in proteins solubility and a possible enhancement of their emulsifying ability as well as desirable changes in the physicochemical properties of oil-water interfacial layer [Demetriades & McClements, 2000; Kong *et al*., 2001; Diftis & Kiosseoglou, 2004]. In contrary, the unclear dependence between emulsion stability and HLB of added surfactants in emulsions C2-C7 may be related to the fact that in some of the samples the interactions between all constituents of emulsifying mixtures were probably correlated negatively [Dickinson & Merino, 2002; Dalgleish, 2006].

Influence of emulsion composition on the relative retention of diacetyl and (-)-α-pinene

In all investigated samples there were detected positive percentage values of relative retention, which means that hydrophilic diacetyl and hydrophobic (-)-α-pinene were retained by the emulsions (Figure 1AB). Moreover, ANOVA revealed that natural emulsifiers affected more than surfactants the vapor-matrix partition coefficients (K_{matrix}) of (-)-α-pinene and diacetyl (Table 5). This may suggest that odorants could have interacted – to a greater extent – with natural surface-active components than with surfactants. Figure 1AB shows that in general, hydrophobic aroma compound demonstrated higher relative retention than hydrophilic diacetyl. This could be associated with hydrophobicity and solubility of the studied aroma compounds in the aqueous and organic solvent phases. The hydrophobic aroma compound with log P =4.83 (Table 1) was mainly dissolved in the internal phase of the emulsions, and to be released in the headspace it had first to diffuse from oil droplets to the aqueous phase, which generally inhibits this process [de Roos, 1997; Druaux & Voilley, 1997]. Almost in all samples prepared with 2 wt% natural emulsifier the relative retention was observed to be higher than in the respective samples prepared with 1 wt% this component, irrespective of aroma compound hydrophobicity. The highest values of 73.4% and 92.9% for diacetyl and (-)-α-pinene, respectively, were recorded in samples marked A1 (Figure 1AB). Proteins are large complex amphipathic molecules containing combinations of ionic, polar and non polar regions, which makes them surface-active and strongly interacting with many other food components [Fischer & Widder, 1997; Dickinson, 1999]. Interactions between diacetyl and proteins of sodium caseinate that resulted in increased retention of the studied hydrophilic aroma compound were reported by Landy *et al*. [1995] and Lubbers *et al*. [1998]. Moreover, some other reports indicate the binding of diacetyl to raw egg albumin [Leland, 1997] and to gelatin [Bakker *et al*., 1998]. Furthermore, evidence also exists for the binding of carbonyl flavor compounds to soy proteins and β-lactoglobulin [O'Keefe *et al*., 1991; Andriot *et al*., 2000]. These findings may suggest the activity of diacetyl [Rankin & Bodyfelt, 1996] for physicochemical interactions with proteins and may explain its higher retention observed in the samples made with 2 wt% than in those formed with 1 wt% natural emulsifier. The enhanced (-)-α-pinene retention detected between samples A2--A1 and C2-C1 may be related to the higher surface protein concentration (Table 3). Proteins are able to form a con-

TABLE 5. ANOVA table of the influence of natural and synthetic emulsifiers on vapor- matrix partition coefficients (K_{matrix}) of diacetyl and (-)-α-pinene in emulsions.

Aroma compound	Natural emulsifiers (NE)		Synthetic emulsifiers (SE)		(NE × SE)	
	F	df	F	df	F	df
Diacetyl	66.31***	2	12.75***	4	24.51***	8
(-)-α-Pinene	2064.84***	2	468.49***	4	24.40***	8

Significance level: ***α≤0.001.

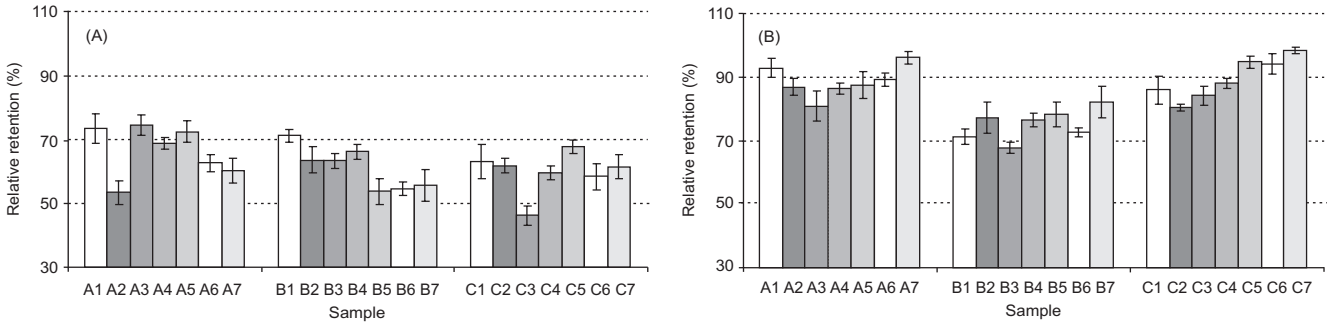

FIGURE 1. Effect of emulsion composition on the relative retention of diacetyl (A) and (-)-α-pinene (B).

densed viscoelastic film of immobile, highly self-interacting molecules in the interface [Cornec *et al*., 1996; Druaux & Voilley, 1997]. Moreover, adsorbing at the oil-water interface proteins can unfold and rearrange their secondary and tertiary structure to expose hydrophobic residues to the hydrophobic phase, which could have also enhanced their interactions with the studied hydrophobic aroma compound [Phillips, 1981; Wilde *et al*., 2004]. It shall also be stressed that volatility of (-)-α-pinene in the samples containing dried egg yolk could be affected by lipids (phospholipids and cholesterol) from lipoproteins (HDL and LDL) [Anton *et al*., 2000]. The correlation coefficients calculated between relative retention of aroma compounds and protein concentration in the samples containing only natural emulsifier (A1, A2, B1, B2, C1, C2), showed positive but relatively low values (Table 6). The positive correlation values may suggest that different interactions occurred, whereas the low ones probably resulted from different physicochemical properties of the proteins such as surface hydrophobicity [Philips, 1981; Fisher & Widder, 1997; Aluko & Mine, 1998]. Addition of surfactants, with HLB value lowering from 40 to 11, to the emulsions containing 1 wt% natural emulsifier (A3-A7, B3-B7 and C3-C7) generally increased relative retention of (-)-α-pinene, whereas regarding diacetyl, changes of this parameter were clearly dependent on surfactant type (Figure 1AB). The highest retention for diacetyl of 74.3% was found in sample A3, whereas the lowest one of 46.2% in emulsion C3. Regarding (-)-α-pinene the highest (98.4%) and the lowest (67.7%) retention values were identified in samples C7 and B3, respectively. Significant correlation values between retention and HLB were noticed for diacetyl in samples A3-A7 ($r=0.875$, $\alpha\leq0.05$), whereas regarding hydrophobic odorant in samples: A3-A7 ($r=-0.925$, $\alpha\leq0.05$) and C3-C7 ($r=-0.959$, $\alpha\leq0.01$) (Table 6). These results show an opposite effect of HLB on diacetyl and (-)-α-pinene retention in samples A3-A7 and B3-B7 and may suggest that diacetyl requires more hydrophilic emulsifiers to increase retention. It was also found that displacement of proteins from oil-water interface by surfactants did not affect retention of (-)-α-pinene, because with lowering surface protein concentration retention of this aroma compound increased (Table 3 and Figure 1B). To explain these phenomena various cases of coexistence of proteins and surfactants have to be taken into consideration. Dickinson *et al*. [1999] suggested that the behaviour of mixed systems containing anionic surfactants was strongly related to the presence of surfactant-protein association structures and interfacial interactions. In addition, Malhotra & Coupland [2004] reported that charged surfactants were capable of maintaining a pH-independent negative charge on the proteins so their complexes could be highly soluble even at the isoelectric point. This may explain the enhanced interactions between charged groups of diacetyl and proteins in the samples containing SDS (A3-A5, B4 and C5) [Rankin & Bodyfelt, 1996]. Moreover, Fang & Dalgleish [1996] demonstrated that SDS may promote polymer formation of β-casein contributing to physical characteristics of interfacial layer, which could be associated with an increase of retention of (-)-α-pinene observed in sample A5. Furthermore, Aluko & Mine [1998] showed that treatment with SDS caused the lipoproteins of egg yolk to unfold and expose part of the lipid held within the molecule, which may be related to the increase of (-)-α-pinene retention in emulsions C3-C5 containing SDS and egg yolk (Figure 1B). On the other hand, Stauffer [2001] and Capek [2004] reported that the unadsorbed proteins and surfactants can form in the bulk phase micelles with relatively high hydrophobic cavity. In addition, van Ruth *et al*. [2002b] suggested affinity of hydrophobic odorants including (-)-α-pinene to the hydrophobic cavities formed by Tween 20 in water phase as well as an increase of retention of hydrophilic aroma compounds caused by higher micelle concentrations in the water phase or inverse micelles formed in the oil phase. Summarizing, it may be supposed that the process of micelle formation and specific interactions between investigated flavour compounds and proteins as well as other surface-active components may have contributed to the increase of odorants retention. However, regarding diacetyl interactions with water molecules such as H-bonding or van der Waals interactions as well as interactions with lecithins it probably also affected its retention [Rankin & Bodyfelt, 1996; Seuvre *et al*., 2006].

TABLE 6. Correlation values between relative retention of diacetyl or (-)-α-pinene and protein concentration as well as hydrophilic-lipophilic balance (HLB) of surfactants.

Aroma compound	Protein concentration[a]	HLB[b]	HLB[c]	HLB[d]
Diacetyl	0.669	0.875*	0.781	-0.639
(-)-α-Pinene	0.422	-0.925*	-0.705	-0.959**

Significance level: *$\alpha\leq0.05$; **$\alpha\leq0.01$; [a, b, c, d]Calculated between emulsions: A1, A2, B1, B2, C1, C3 and A3-A7, B3-B7, C3-C7, respectively.

Influence of microstructural properties, stability and viscosity of emulsions on release characteristics of diacetyl and (-)-α-pinene

Figures 2AB and 3AB illustrate the values of release rate constant and release mechanism of diacetyl and (-)-α-pinene, respectively. It was observed that the decrease of concentration from 2 to 1 wt% of natural emulsifier generally increased release rate constants of the studied odorants, particularly sharply in the samples flavoured with (-)-α-pinene (Figure 3A). These results are consistent with those reported by Druaux & Voilley [1997] and Rogacheva *et al*. [1999] and may suggest that poor coverage with proteins on the surfaces of the fat globules probably decreased the resistance to mass transfer of (-)-α-pinene from oil droplets to the water phase [Khwaldia *et al*., 2004]. Moreover, the higher release of (-)-α-pinene that was registered between emulsions A1-A2 and C1-C2 may have been due to increase of interfacial area, which could induce higher mass transfer of this aroma compound (Figure 3A and Table 3). In all samples negative correlation values were registered between release rate constants and viscosity (Table 7). Significant correlation values between these parameters regarding diacetyl were observed in all studied samples, whereas for (-)-α-pinene only in the samples formed with surfactants and/or sodium caseinate (A1-A7). The results confirm those reported by Rankin & Bodyfelt [1996], Charles *et al*. [2000] and Terta *et al*. [2006] and suggest that viscosity was an important parameter that reduced diffusion particularly of hydrophilic flavour molecules in the emulsions. Surfactants added to the emulsions generally affected release of the studied flavor compounds however, significant positive correlation values ($\alpha \leq 0.05$) between release rate constants and decreasing HLB value were found only for (-)-α-pinene in samples: B3-B7 ($r=0.903$) and C3-C7 ($r=0.899$) (Table 7). These findings suggest that the release of hydrophobic aroma compound was to a greater extent dependent on the hydrophilic-lipophilic balance of surfactants than the one of diacetyl. Stability of the emulsions measured towards creaming significantly ($\alpha \leq 0.05$) affected diacetyl release in samples A1--A7 and C1-C7, however expected negative values ($r=-0.804$) were found only in emulsions A1-A7. Regarding (-)-α-pinene, a negative correlation value ($r=-0.783$, $\alpha \leq 0.05$) was recorded in samples B1-B7 (Table 7). The studies also showed that microstructural properties of the emulsions *i.e.* surface protein concentration, specific surface area and dispersion index, had no significant effect on (-)-α-pinene release (Tables 3 and 7). The results partly confirm those demonstrated by Landy *et al*. [1996] and are in contrast to the effects demonstrated by van Ruth *et al*. [2002a]. The Avrami's parameter n of diacetyl and (-)-α-pinene, in all studied samples was lower than 1 (Figures 2B and 3B), which suggests that the molecular diffusion was limiting the rate of release of the investigated aroma compounds [Soottitantawat *et al*., 2004].

Correlations between odor intensity and aroma headspace concentration as well as viscosity and stability of the emulsions

Generally, the values of odor intensity reported by assessors by orthonasal perception were positively correlated with the appropriate concentration of aroma compounds measured by gas chromatography-mass spectrometry (Table 8). Significant correlation values ($\alpha \leq 0.05$) were found for diacetyl ($r=0.869$) in emulsions C1-C7, whereas regard-

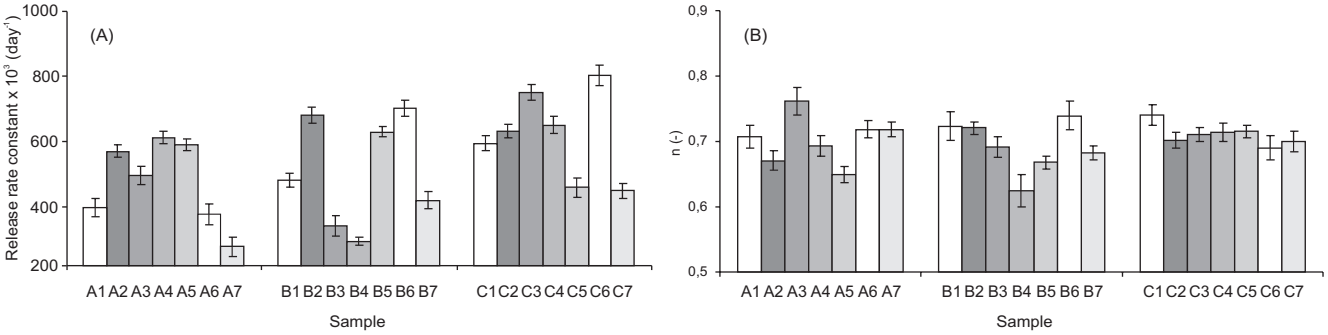

FIGURE 2. Effect of emulsion composition on the release rate constant (A) and the release mechanism (B) of diacetyl.

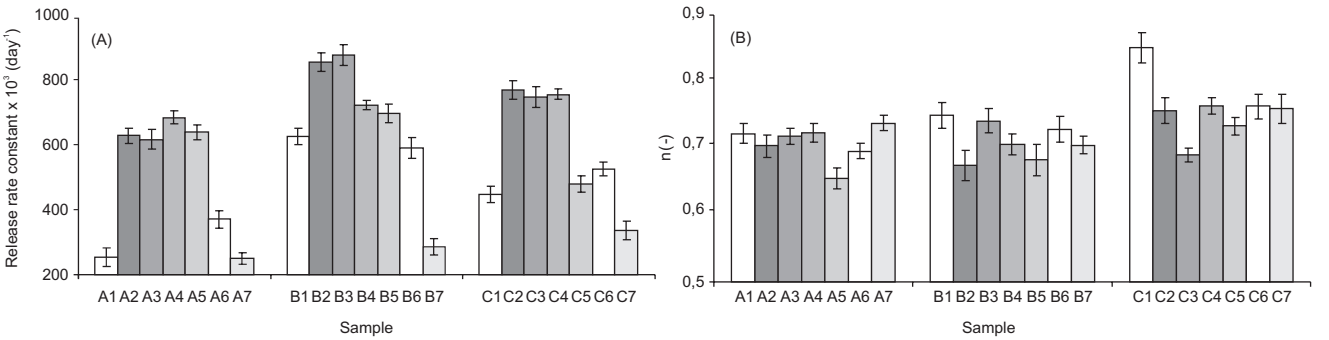

FIGURE 3. Effect of emulsion composition on the release rate constant (A) and the release mechanism (B) of (-)-α-pinene.

TABLE 7. Correlation values between release rate constants of diacetyl or (-)-α-pinene and viscosity, hydrophilic-lipophilic balance (HLB), creaming stability (ES), specific surface area (SSA) and dispersion index (DI).

Code	Viscosity		HLB[a]		ES		SSA	DI
	diacetyl	(-)-α-pinene	diacetyl	(-)-α-pinene	diacetyl	(-)-α-pinene	(-)-α-pinene	(-)-α-pinene
A1-A7	-0.827*	-0.813*	0.689	0.827	-0.804*	-0.575	-0.248	-0.591
B1-B7	-0.918**	-0.306	-0.576	0.903*	-0.343	-0.783*	-0.443	-0.165
C1-C7	-0.909**	-0.621	0.406	0.899*	0.776*	0.489	0.656	-0.251

[a]Correlation calculated for emulsions: A3-A7, B3-B7 and C3-C7; significance level: *≤0.05; **α≤0.01.

TABLE 8. Correlation values between odor intensity and headspace concentration of diacetyl or (-)-α-pinene, viscosity and creaming stability of the emulsions.

Code	Concentration		Viscosity		Creaming stability	
	diacetyl	(-)-α-pinene	diacetyl	(-)-α-pinene	diacetyl	(-)-α-pinene
A1-A7	0.303	0.619	-0.822*	-0.782*	-0.781*	-0.761*
B1-B7	0.636	0.822*	-0.826*	-0.462	-0.329	-0.514
C1-C7	0.869*	0.859*	-0.522	-0.836*	0.322	0.861*

Significance level: * α≤0.05.

ing (-)-α-pinene (r=0.822 and r=0.859) in samples B1-B7 and C1-C7, respectively (Table 8). These results indicate that assessors had more problems to correctly detect odor intensity of diacetyl than (-)-α-pinene. This could be associated with sensory properties of diacetyl which is very odorous and its saturated vapour pressure is relatively very high [Seuvre *et al*., 2006]. Moreover, the release rate of (-)-α-pinene was probably slower than that of diacetyl due to the fact that hydrophobic aroma compound was released from the internal phase of the emulsions and that its molar volume (207.2 cm^3/mol) is higher than the one of hydrophilic odorant (96.2 cm^3/mol) (Table 1). In all emulsions there were registered negative correlation values between odor intensity and viscosity, which indicates that with increasing viscosity of the samples assessors detected lower intensity of investigated aroma compounds (Table 8). Significant correlation values (α≤0.05) between odor intensity and viscosity regarding diacetyl were found in emulsions A1-A7 (r=-0.822) and B1-B7 (r=-0.826), whereas for (-)-α-pinene in samples: A1-A7 (r=-0.782) and C1-C7 (r=-0.836) (Table 8). The relatively good relationship between odor intensity detected by assessors and viscosity of the emulsions may be explained by the fact that viscosity is the most important parameter that influences the value of mass transfer coefficient of aroma compounds in liquid systems [Harrison *et al*., 1997]. The correlation coefficients calculated between odor intensity and stability of the emulsions measured towards creaming showed only small dependence between these two parameters, because significant (α≤0.05) negative correlation values were found only in samples A1-A7 for both studied aroma compounds.

CONCLUSIONS

The studies showed that retention and release of diacetyl and (-)-α-pinene were dependent on many factors such as: mass transfer, matrix structural hindrance and aroma compound-matrix interactions. The natural emulsifiers showed a profound effect on the retention and release characteristics of both investigated odorants. The hydrophobic interactions between studied aroma compounds and proteins as well as micelles formation probably mainly contributed to the increase of relative retention of diacetyl and (-)-α-pinene. Viscosity of the emulsions mostly affected diacetyl release, whereas regarding (-)-α-pinene this process was significantly influenced by hydrophilic-lipophilic balance (HLB) of surfactants. The microstructural properties, *i.e*. specific surface area, surface protein concentration and droplet diameter, did not influence significantly the release of (-)-α-pinene, which may suggest complementary investigations in simpler systems to better understand the influence of these factors. Sensory analyses of odor intensity showed higher correlation values with respective data received from instrumental analyses regarding (-)-α-pinene than diacetyl.

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