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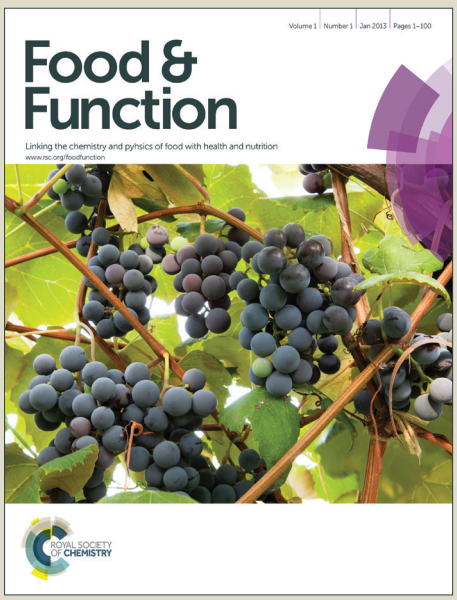

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Chemical stability of lemon oil components in sodium caseinate–lactose glycoconjugates–stabilized oil–in–water emulsions based on using solid-phase microextraction-gas chromatography

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A headspace solid–phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC/MS) was developed to quantify lemon oil components and their degradation products in oil-in-water (O/W) emulsions prepared with sodium caseinate-heated-lactose (NaC-T+Lact) glycoconjugates as wall material at two pH values (3.0 and 6.8). NaC-T+Lact conjugates had a significantly lower solubility at both pHs. Hydrolysis prior to glycation enhanced the solubility of the glycoconjugates. Glycation with lactose did not improve the emulsion activity of NaC, while caseinate glycoconjugates showed much stronger antioxidant activity than NaC-control sample. This might be due to presence of melanoidins formed between the sugar and amino acid compounds as supported by the increase in browning intensity. Among the SPME-fibers tested, carboxen/polydimethylsiloxane (CAR/PDMS) provided better results in term of sensitivity and selectivity for oil lemon components and their degradation products. Storage studies of these emulsions demonstrated that glycated NaC-T+Lact showed protection against peroxidation compared to control. However, acidic pH conditions altered their stability over storage time. The major off-flavor components (α-terpineol and carvone) were inhibited in emulsions stabilized with glycated NaC, particularly at pH 6.8. The use of NaC-T+Lact conjugate showed improved encapsulation efficiency and stability and could be used as potential food ingredient-emulsifiers for stabilising citrus oils against oxidative degradation in foods and beverages application.

Introduction

Consumers demand for citrus juice of fresh quality is increasing. However these drinks or concentrates deteriorate rapidly due to the formation of off-flavors and loss of typical flavor intensity during storage. Essential oil such as lemon oils are rapidly degraded since they are prone to chemical degradation when exposed to harsh environment.[1] The high level of unsaturated and oxygen functionalized terpenes render lemon oil very susceptible to oxidation. Citrus oil is a mixture of volatile compounds and consists mainly of monoterpenes hydrocarbons, which possess high levels of unsaturation and are generally unstable due to many factors, such as light, oxygen and heat.[2]

Citral is one of the key flavor components in lemon citrus fruits. Unfortunately, citral and other citrus flavor components such as limonene are highly susceptible to acid-catalyzed and oxidative, leading to a loss of fresh citrus flavors. The schematic diagram of the citral degradation pathway is shown in Fig. 1A.[3] At low pH, the first reaction is responsible for the generation of off-flavors such as terpineol and also the loss of desirable lemon impact such as citral. Due to its unsaturated character, d-limonene can yield by-products such as carvone, carveol, 2,8-menthadiol and limonene oxide via radical formation by oxidative pathways. The schematic diagram of the d-limonene degradation pathway is shown in Fig. 1B.[4,5]

Strategies are needed to protect citrus oils from degradation. Several studies have demonstrated that emulsions prepared with multiple layers of emulsifiers have improved stability to environmental stresses. Lemon oil can therefore be stabilized efficiently by encapsulation if appropriate coating materials are well selected. Early products prepared with single polysaccharides commonly used by the food industry still showed flavor defects, as for example the gum arabic capsules which are somewhat permeable. A slight amount of the lemon oil could migrate to the surface of the capsule and become oxidized with subsequent

formation of carvone, 1,2-1imonene epoxide, p-methylacetophenone, 1,2-dihydroxylimonene and p-cymene.[6,7]

Sodium caseinate (NaC) is widely used in the food industry as functional ingredient because of its nutritional value and unique functional properties, namely fat-binding, water-binding, gelation and emulsifying properties.[8,9] To expand its industrial food application, physical, chemical and/or enzymatic modification of the protein has been recommended to modify its configuration, flexibility, hydrophilicity and charge. Among the chemical modification, incubation of various mono, di and polyssacharides (glucose, ribose, lactose, pectines and dextran) with proteins to form glycoconjugates via the "Maillard reaction" is an interesting approach to enhance the emulsifying properties of NaC.[10-14] The improved functional properties of these conjugates are related to the hydrophobic protein being strongly adsorbed to the interface of oil droplets, whereas the bound hydrophilic sugars are strongly solvated by the aqueous phase.[15] The use of Maillard reaction products for encapsulation of oil has been found effective for protecting microencapsulated fish oil and other vegetable oils[16-18] from oxidation, as well as controlling the digestibility of lipids within the human gastrointestinal tract.[19]

Different analytical techniques have been used to isolate and study lemon oil components and their degradation/oxidation products in foods and beverages, particularly in oil-in-water emulsions.[20-23] To study the stability and determine the time dependent degradation of such compounds, the extraction and analytical techniques should not adulterate samples. The solid-phase microextraction (SPME) technique fits food industry flavor analysis criteria, as it is solvent-free, low cost, easy to use and relatively fast, yet sensitive enough for quality control purposes and does not adulterate samples at suitable extraction temperatures. Methods have been developed in our laboratory to optimize the analysis of volatile

compounds by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC/MS) [24,25] that will be adapted to study lemon oil components degradation/oxidation in oil-in-water (O/W) emulsions encapsulated with NaC-Lact glycoconjugates. The objectives of the present study were to develop a HS–SPME–GC/MS method for the analysis of lemon oil components and monitor their time dependent degradation in O/W emulsions prepared with NaC and NaC-Lact glycoconjugates as wall materials at two pH values (3.0 and 6.8).

Materials and methods

Chemicals and reagents

All standards (d-limonene, β-pinene, cis-citral (neral), trans-citral (geranial), terpineol and carvone) and reagents used in this study were purchased from Sigma-Aldrich (Oakville, ON, Canada). Solvents were purchased from Burdick & Jackson and supplied by VWR International (Toronto, ON, Canada). SPME fibres (carboxen/polydimethylsiloxane [CAR/PDMS, 85 μm], polyacrylate [PA; 85 μm] and carbowax/divinylbenzene [CW/DVB; 65 μm]) were purchased from Supelco (Oakville, ON, Canada). The commercial ready to use flavoring/clouding agent for lemon drink consisted of a mixture of Neobee-M5 as clouding agent, Sucrose acetate isobyturate (SAIB) as weighting agent and a cold pressed 5-Fold Lemon oil from California as flavoring agents (composition 52.8% of limonene and 15.0% of citral) was generously donated by Flavorcan International Inc. NaC was purchased from Fonterra Ltd (Auckland, New Zealand, protein 90.4%, ash 3.8%, fat 1.1%, lactose 0.1%). The NaC was stored at 4 °C and used without further purification. Cumene hydroperoxide (CH, 80% pure), hydrochloric acid, sodium hydroxide and enzyme were obtained from Sigma-Aldrich. Double distilled water was used to prepare all the solutions.

Preparation of standards

For qualitative analysis, standard solutions were prepared by dissolving 1 µL or 1 mg of each individual standard in 10 mL of methanol. Individually and as a mixture, 10 µL of standard solution was diluted in 1 mL of NaCl solution (6 M), disposed into 15 mL glass vials (Supelco, Oakville, ON, Canada), closed using a Teflon/Silicone (TEF/SIL) septum and analyzed by SPME-HS-GC/MS. A gas tight syringe was used for sample preparation to minimise loss.

For quantitative analysis of each compound, calibration curves were built for the CAR/PDMS fibre procedure by analysing, in duplicate, 10-µL of formulation samples (containing all the ingredients present in the emulsion, except lemon oil) spiked with a mixture of targeted oil components (d-limonene, β-pinene, cis-citral and trans-citral) and degradation product (terpineol and carvone) at concentrations ranging from 0.5 to 100 µg mL^{-1}.

Preparation of samples

Maillard conjugates. NaC (120 g) and lactose (44.85 g) were dissolved in distilled water to give an 8% (w/v) protein solution. The molar ratio of epsilon amino acid to sugar carbonyl was 1:1.5. Two different conjugates were prepared as follows: For the first conjugate (NaC-T+Lact = sodium caseinate-heated-lactose), the protein solution was adjusted to pH 7.5 (pH-meter 240 Corning) with diluted HCl or NaOH, stirred, heated at 80 ^{o}C for 20 min in a water-bath, then cooled down at 21 ^{o}C prior to the addition of lactose sugar. For the second conjugate (NaC-T/E+Lact = sodium caseinate-heated/hydrolyzed-lactose), the sodium caseinate was first heated at 80 ^{o}C for 20 min then cooled down at 37.5 ^{o}C, the protein solution was adjusted to pH 7.5 prior to the addition of trypsin enzyme at 0.25% w/w of protein (Type IX-S, Sigma T0303), for a hydrolysis period of 0 to120 min. The hydrolysis was monitored using a pH-stat apparatus (TIM 865, Radiometer Analytical SAS, Villeurbanne, France) under controlled conditions for pH, temperature, and stirring speed.

Aliquots were collected at 0, 30, 60, 90 and 120 min to determine the degree of hydrolysis. After 120 min, the hydrolysis reaction was terminated by heating the sample at boiling temperature for 10 min. The degree of hydrolysis was determined using the o-phthaldialdehyde (OPA) reagent according to Church *et al.*[26] After the completion of the hydrolysis, the solution was adjusted to 21 ^{o}C followed by the addition of lactose sugar. The treated samples were then submitted to freeze-drying. The freeze-dried samples (approx. 15 g each) were placed in aluminium dishes, and incubated at 50 ^{o}C in sealed desiccators maintained at 65% relative humidity with saturated aqueous potassium iodide (KI) solution.[27] The samples were incubated for 24 hours (Reach-in incubator Forma Scientific Model 3940) in separated desiccators and then stored at –20 ^{o}C until further analysis. A third control sample of the NaC without any sugar added (NaC-T-control) was treated similarly.

Physicochemical and functional analysis. Protein solubility at both pH levels was determined by the method of Betschart [28] with some modifications. 100 mg of protein sample was dispersed in 10 mL of water; the pH was adjusted to the desired level using 1 N HCl or 1 N NaOH. The dispersion was stirred continuously for 30 min and centrifuged at 3000 rpm for 30 min. The supernatant was recovered, and the amount of protein in the supernatant was determined by the method of Bradford.[29] Solubility was calculated as the percent ratio of protein in the supernatant to that of the total protein in the initial sample. Emulsifying activity index [EAI] was analyzed using the method of Pearce and Kinsella.[30] Color measurements (L*, a*, b*) of glycoconjugate powders were measured using the Hunterlab (Labscan tristimulus spectrophotometer VI-A30, Hunter Associates Lab., Inc., Reston, VA, USA) following the method of Chantrapornchai *et al.*[31]. ΔE (colour difference) values were calculated using the following formula: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$, where ΔL, Δa and Δb are the differences in the specified tristimulus coordinate between the sample and sodium caseinate used as control. Free radical-scavenging activity (DPPH) of glycoconjuagtes was

166 estimated according to a modified procedure reported of Cämmerer and Kroh.[32] Samples
167 (0.8% w/w) were dissolved in distilled water and shaken vigorously for 30 min and
168 centrifuged at 3,750 rpm for 10 min. An aliquot of 200 µL of the supernatant was added to a 1
169 mL solution of DPPH (prepared daily) to give a final absorption of ± 1.8 AU, 74 mg/L in
170 ethanol, put in dark x 1 h, mixing, and then measured at 520 nm using blank ethanol. The
171 DPPH concentration in the reaction medium was calculated from the following calibration
172 curve, determined by linear regression; $[DPPH]_t$ = 0.0296 (Abs-520 nm) - 0.0086 (r^2=
173 0.9999), where $[DPPH]_t$ was expressed as mg/L. The antiradical activity of sample was
174 expressed as percentage disappearance of DPPH; the greater the percentage disappearance of
175 the initial purple colour, the greater is the antiradical activity. The percentage of remaining
176 DPPH (%DPPHrem) was calculated as follows; $\%DPPH_{rem} = ([DPPH]_t/[DPPH]_o) \times 100$,
177 where $[DPPH]_o$ is the concentration at time zero.[33]

178 **Preparation of oil-in-water emulsions.** Oil-in-water emulsions were prepared by
179 mixing 10% oil (98.0% Neobee M5 with sucrose acetate isobyturate (SAIB), 2.0% Lemon oil
180 (composition 52.8% of limonene and 15.0% of citral)) into an aqueous phase consisting of
181 distilled water adjusted to pH 3.0 and 6.8 with NaC-T, NaC-T+Lact and NaC-T/E+Lact
182 (prehydrated overnight at 4 ^oC), at a final emulsifier-to-oil ratio of 1:10 (1.0 wt% protein for
183 10 wt% oil). Prior to the homogenisation step, cumene hydroperoxide (100 mmol kg^{-1} oil),
184 used as a prooxidant, was dissolved in the oil phase and stirred for 15 min. A coarse emulsion
185 premix was prepared by homogenizing oil and aqueous phase using a PT 2100 Polytron
186 homogenizer (Kinematica AG, Littau-Luzern, Switzerland) at setting 8000 r.p.m for 1 min at
187 room temperature. The coarse emulsion was then passed through a valve homogenizer
188 (Emulsiflex-C5, Avestin, Ottawa, ON, Canada) at 3000 psi for 3 passes and 1000 psi for 1
189 pass. All emulsions (10 mL, in triplicates) were stored in the dark in 15 mL amber vials at 55
190 ^oC for various period of time (0 to 35 days).

HS–SPME–GC/MS analyses. A 10-μL of emulsion was sampled and added to a 1 mL of NaCl solution (6 M) into a 10-mL screw-top headspace amber vial; the vial was sealed with a magnetic screw cap containing a polytetrafluoroethylene (PTFE)/silicone septum (Varian, Mississauga, ON, Canada).

The HS–SPME sampling was carried out using an automated multipurpose sampler (MPS2; Gerstel, Baltimore, MD, USA). The fibre coating was exposed to the emulsion headspace for 45 min at 45 ^{o}C. Targeted compounds were desorbed during 3 min by directly inserting the fibre into the injection port of the gas chromatography (GC) unit, which ran in splitless mode for 3 min at 300 ^{o}C. The same fibre was used for all the analyses (around 100 injections).

The analyses were performed using a Varian model 3800 GC system fitted with a 1078/1079 split/splitless injector (Glass insert SPME, 0.8 ID; Varian, Mississauga, ON, Canada) suitable for HS–SPME analysis, along with a Saturn 2000 mass spectrometry (MS) system. Helium was used as the carrier gas with a constant flow rate of 1 mL min^{-1}. The components were separated on a VF-5ms (5% phenyl/95% dimethylpolysiloxane) capillary column measuring 30 m × 0.25 mm with a film thickness of 0.25 μm. The oven temperature program began with 3 min at 35 ^{o}C, followed by a 6 ^{o}C min^{-1} increase to 80 ^{o}C, a 20 °C min^{-1} increase to 280 ^{o}C, and 2 min at 280 ^{o}C. Detection was carried out by MS on the total ion current obtained by electron impact at 70 eV. The mass range acquisition was m/z 30–200. The standards were injected and identified by means of their retention times (RTs) as well as with searches of the 2005 version of the NIST Mass Spectral Library.

Optimization of SPME procedure and fiber selection. To determine the main parameters influencing the sensitivity of the HP-SPME method, the effects of fiber type (PDMS–DVB, 85 μm; PA, 85 μm; CW–DVB, 65 μm0), incubation time (30–60 min), incubation temperature (30–65 °C), NaCl concentration (0-6 M) and sample volume (10–50

µl) were studied. The assessment was based on MS responses of selected compounds. An experimental design with two levels (2^k tests, k: number of factors) was set up in duplicate for each fibre; for each chosen parameter, two values (low, high) were fixed as an experimental field. The most significant parameters were then selected to generate a central composite design in order to build a predictive model (response surface model: RSM) of the MS responses. Then, the factors studied were chosen for each fibre so as to obtain the maximum sensitivity for all compounds.

Statistical analysis. Data were statistically analyzed by one-way analysis of variance (ANOVA) using the PRISM software, version 3.02 (Graph Pad Software, Inc. San Diego, CA, USA). Significant differences between means were determined by Tukey's Multiple Comparison Test procedure at the 5% significance level.

Results and discussion

Selection of SPME fibre

The concentration of analytes in the headspace depends in general on several factors: 1) the concentration in the original sample; 2) the volatility of the compound; 3) the solubility of that compound in the sample matrix; 4) the temperature of incubation; and 5) a combination of the sample volume and the time of incubation.[34]

Based on their capacities to show broad retention over a wide range of polarity, three types of fibers had been selected: CAR/PDMS (85 µm), PA (85 µm) and CW/DVB (65 µm). Fig. 2 shows comparative response of the three SPME fibers for the selected volatile compounds under same running conditions. Of the three fibers, our results showed that the total ion current of all targeted compounds were 205, 90 and 188 for CAR/PDMS, PA and CW/DVB fibres, respectively. In terms of sensitivity and selectivity, CAR/PDMS provided better results than the others for oil lemon components and their degradation products, and

240 was thus selected for subsequent work. The stronger response of the CAR/PDMS matrix,
241 which is rich in micropores is efficient at adsorbing gases, trace-level volatiles and low
242 molecular weight compounds.[35] The efficiency of the CAR/PDMS fiber was concurred with
243 other studies on foodstuffs.[36] In fact, this fibre showed the greatest capacity to extract
244 chemical compounds with a broad spectrum of polarities and molar masses.[37]

245 **Optimization of HS–SPME–GC/MS parameters**

246 The temperature and the time of extraction and desorption were reported previously to be the
247 most significant factors in the HS–SPME–GC/MS analysis of flavor compounds.[38] Factors
248 modifying the matrix can also influence the sensitivity of the fibre extraction. The addition of
249 a salt such as NaCl improves the effectiveness of the extraction by decreasing the solubility of
250 the analytes (phenomenon of salting out) in an aqueous sample.[38] The pH can also modify the
251 matrix; for example, the use of a 0.1 M phosphate buffer, with a pH lower than the pK_a of the
252 acids involved, decreases the solubility of the acids and renders them more volatile.[39] Finally,
253 sample agitation reduces the extraction time and generally improves extraction efficiency.[39]
254 Those parameters had all been considered in a previous study [25] and were taken into account
255 in the present one as well. The following extraction time (30 min, 45 min and 60 min) and
256 temperature (30 ^{o}C, 45 ^{o}C and 60 ^{o}C) values have all been tested in all combinations using
257 CAR/PDMS fibre. An extraction time of 45 min at 45 ^{o}C showed the best sensitivity, in terms
258 of peak intensity, specifically for lemon oil components.

259 Matrix preparation and sample volume can also strongly influence the adsorption of
260 analytes onto the SPME fibre. At higher values for each of these parameters, reverse diffusion
261 of analytes could occur from the fibre to the sample, resulting in a reduction of the fibre's
262 capacity to adsorb the analytes.[40] A series of tests were carried out on selected fibre in spiked
263 formulation with and without the addition of 1 mL of Milli-Q water or NaCl solution (6 M).
264 These treatments evaluated the influence of the addition of water or salt on the migration of

265 the analytes from the matrix to the headspace. The addition of NaCl solution (6 M) and
266 sample agitation were found to be more efficient during extraction, specifically for polar
267 compounds such as carvone and terpeniol. A 10-µL volume of emulsion was sufficient to
268 allow detection of targeted compounds; only a factor of 1.7 fold was observed when using 50
269 µL of emulsion, this could be due to the fibre saturation. Desorption time was set at 3 min as
270 lower time was not sufficient to completely desorb some analytes.

271 **Method validation**

272 Calibration curves were prepared by plotting the average peak areas of the standard solutions
273 against the corresponding concentrations. Then the curve characterized by slope (b), intercept
274 (a), and correlation coefficient (R^2) was used to determine the concentration of the targeted
275 compound in the analyzed samples. The relationship of peak area and concentrations were
276 linear for all compounds and the range of linearity for each compound is indicated in Table 1.

277 **Detection limits (DL) and quantification limits (QL).** The detection limit (DL) was
278 assumed to be less or equal three times Signal/Noise (DL≤3 S/N; Table 1). The quantification
279 limit (QL) was assumed to be less or equal ten times Signal/Noise (QL≤10 S/N).

280 **Repeatability.** The precision was also assessed from calibration curve of each studied
281 compound for CAR/PDMS fibre by determining the intra-assay coefficients of variation,
282 which measure the variability of the results for the same sample evaluated repeatedly in the
283 same assay (run). It is generally agreed that intra-assay variability of less than 10% to 15% is
284 acceptable.[41] In the present study, the repeatability (precision) of extraction by the
285 CAR/PDMS fibre was measured with six independent emulsion samples containing lemon oil
286 and spiked with 1 µg for each degradation product. The relative standard deviations (%RSD)
287 ranged from 6.3 to 14.8 %, with a mean of 9.1 % across targeted compounds (Table 1). These

values were satisfactory and in agreement with other studies on various matrices and different volatiles.[34]

Physicochemical and functional properties.

Solubility. It is well established that the solubility of NaC in solution is influenced by pH; at neutral pH sodium caseinate showed higher % solubility compared to pH 3 which is near the isoelectric pH of the caseins (pI ~ 4.6). The solubilities of the NaC-T-controls and NaC-T-Lact conjugates, as a function of pH (3 and 6.8), are shown in Table 2. The NaC-T-Lact conjugates had a significantly lower solubility of 38.9% and 59.9% at pH 3 and 6.8, respectively; representing a loss of 36.5% of their original values. This significant decrease in solubility of conjugated NaC, can be attributed to the polymerization and further cross-linking of protein molecules. These results were in good agreement with published literature on protein-saccharide conjugation reactions.[42-44] Enzymatic hydrolysis treatment of caseinate proteins prior to lactose glycation resulted in lower molecular weight polypeptides with enhanced solubility. Nac-T/E-Lact glycoconjugates lost only 4.7% and 6.6% in solubility compared to their unglycated counterparts at pH 3 and 6.8, respectively. The results indicated that the solubility of the NaC-T-Lact conjugates was pH-dependent, and therefore might have an effect on their emulsifying properties.

Emulsifying properties. The effectiveness of food proteins as emulsifiers is commonly measured and expressed as emulsifying activity index (EAI), which is calculated from the turbidity of an emulsion at a single wavelength.[45] The emulsifying properties of control and glycated sodium caseinate at pH 6.8 are presented in Table 2. EAI of NaC-T-control decreased following glycation and slightly re-increased for the combined heating/ hydrolysis glycated sample. However, these differences were not significant. Groubet *et al.*[46] showed that glycation of caseinate under wet conditions does not improve emulsifying

properties in terms of spectroturbidimetric evaluation of the emulsifying activity index. In dry-heated caseinate–carbohydrate blends, Shepherd *et al.*[47] observed an increased emulsifying activity of caseinate–maltodextrin blends by turbidimetric analysis at pH 4.8, but not at pH 6. The authors conclude that the non- improvement in emulsification properties could be due to steric stabilisation or polysaccharide entanglement (polymerisation).

DPPH-radical scavenging activity. Scavenging of DPPH-radicals is the basis of a common antioxidant assay. Antioxidants can protect against the damage caused by free radicals that have been implicated in the etiology of large number of major diseases.[48] As shown in Table 2, the antiradical activity of our samples was expressed as percent remaining of DPPH; the greater the percentage of remaining DPPH, the lowest antiradical activity. Therefore, our results indicated that glycation significantly reduced remaining %DPPH from 99.2% to 96.5%, meaning that more DPPH radical was scavenged by glycated sodium caseinate with lactose showing much stronger antioxidant activity than NaC-control sample. It has been suggested that the browning compounds formed during the Maillard reaction, which are primarily composed of melanoidins, are major contributor to the radical-scavenging capacity.[49] Thus the radical scavenging activity is correlated to the browning intensity to some extent. It can be found that our results were in line with the browning intensity results. These results were also in agreement with those who reported that the DPPH scavenging activity correlated with the browning intensity.[50,51] Recently, Joubran *et al.*[52] reported also a marked increases in antioxidant capacity of Maillard conjugates as a function of reaction time, protein:monosaccharide mole ratio and moiety type, compared to unglycated protein.

Color development. Color measurements (ΔE) are presented in Table 3. In general, the lower the ΔE, the whiter the color of the sample. Our results indicated that browning was observed following conjugation indicating the formation of Maillard reaction products.

Hydrolysis prior to glycation resulted in more available free amino groups for lactose glycation. This resulted in enhancing significantly the color development (higher ΔE) of Nac-T/E-Lactose compare to control sample. Color formation is likely due to presence of melanoidins formed between the sugar and amino acid compounds.

Degradation of lemon oil components in encapsulated emulsions

HS–SPME–GC/MS method was applied to follow the compositional changes of lemon oil components in emulsions encapsulated with NaC-Lact conjugates during storage in order to better quantify the impact of glycation on the key flavor volatiles and their degradation. Combination of both the heat treatment and hydrolysis of the protein via enzyme addition promote the lysine, arginine and histidine exposure on the protein structure and consequently might increase Maillard reaction to develop conjugates with better interfacial properties, thus resulting in the formation of stronger protein conjugate membranes around fat droplets that better prevent oil oxidation. In the present study, the results indicated a gradual increase in the degree of hydrolysis from 5.71% to 8.35%, 9.14% and 9.29% after 30 min, 60 min, 90 min and 120 min, respectively. Higher the degree of hydrolysis, more ε-amino groups of lysine are accessible for lactose glycation.

It should also be noted, that the degradation of the flavor components in lemon oil quantified by HS–SPME–GC/MS over storage period would be attributed to the deterioration of the emulsion as a whole, independently on the origin (either from the emulsion droplet or from the water phase).

D-Limonene. Limonene is the main component of essential oil present in citrus fruits. The incorporation of lemon oil in beverage needs to be carefully controlled since the terpene derivative such as d-limonene can be degraded by acid catalyzed and autooxidation

359 reactions.[53] The oxidation products of d-limonene include the formation of limonene oxide
360 and carvone.[54] Limonene oxide was not quantified in this study because of its high instability
361 in the emulsion.

362 As shown in Fig. 3, for both the control samples (NaC-T) at pH 3.0 and 6.8, the d-
363 limonene content decreased by 32 and 50% over the 35 days storage period. pH could
364 therefore be considered as a critical variable in emulsion stabilization. In addition, at pH 3.0,
365 the results also indicated that for emulsions stabilized with glycoconjugates NaC-T+Lact and
366 NaC-T/E+Lact, d-limonene degradation was slightly inhibited resulting in 29% and 26% lost
367 respectively, over the storage period. These data may indicate that NaC-Lact glycoconjugates
368 were more effective at retarding d-limonene degradation due to the ability of protein-sugar
369 emulsifier system to form a bulkier polymeric layer than the non conjugated protein on the
370 droplet surface, a kind of antioxidant barrier, thus decreasing prooxidant-lipid interactions. At
371 low pH, emulsion droplets were expected to carry a positive surface charge and thereby repel
372 the transition metal ions present in the aqueous phase and this is most likely a major factor
373 contributing to the increased oxidative stability at low pH.

374 At pH 6.8, limonene degradation was significantly higher (more than 76% loss) in
375 emulsion stabilized with NaC-Lact sample. While, emulsion prepared with NaC proteins
376 subjected to enzymatic hydrolysis prior to lactose glycation offered more efficient protection
377 against d-limonene degradation over the control and non-hydrolyzed glycated samples. In
378 fact, Jahaniaval *et al.*[55] reported that near the pI (pH 3.75 to 4) where the caseins have
379 minimum solubility, the soluble protein fraction exhibited greater hydrophobicity and
380 enhanced emulsifying activity and capacity when compared to higher pHs. Similarly,
381 Ventureira *et al.*[56] reported that the combined effect of the pH of the aqueous phase and
382 enzymatic hydrolysis are determinant factors on the emulsion stability. At acidic pH (pH 2.0)
383 the unfolding and charge of polypeptides and the capacity to form a viscoelastic film at the

384 interface were essential while at alkaline pH (pH 8.0) the balance among high and low
385 molecular mass protein species and flexibility are essential for emulsions properties.

386 **Cis and trans-citral.** Both isomers are the major contributors to the flavor and aroma
387 of lemon oil and represent approx. 15% of the lemon oil constituents.[57] In Fig. 4, the
388 degradation trends of both isomers demonstrated a severe decline at pH 3.0 during the first 2
389 weeks confirming that the acid-catalyzed cyclization exerts a major impact on the
390 deterioration of these two isomers. The use of glycated proteins to ensure protection of these 2
391 isomers had no influence in reducing this rapid decrease of citral under acidic conditions in
392 the emulsion. Similarly, at pH 6.8, the decline for both isomers (cis and trans-citral) was
393 about 60% for both the control and glycated caseinate samples after 35 days accelerated
394 storage conditions. The inhibition of citral degradation was mainly related to the pH of the
395 emulsion solution rather than the effect of glycation. Indeed, as shown by our results (Table
396 2) NaC-control at pH 6.8 showed higher percent solubility than control sample at pH 3.0. Lee
397 *et al.*[45] previously reported a high solubility of sodium caseinated at pH range 6.5-8.0, and
398 minimum solubility around pH 3.5 to 4, which is near the isoelectric points of the casein
399 molecules. Between pH 6.5 and 8, sodium caseinate exists as a polydispersed mixture of four
400 major casein molecules, αs1-CN, αs2-CN (22,000-25,000 kDa), β-CN (24,000 kDa) and κ-
401 CN (19,000 kDa). Heating of NaC combined to enzymatic hydrolysis did not improve the
402 encapsulation efficiency of cis and trans-citral compared to their respective unglycated control
403 samples.

404 **ß-Pinène.** ß-Pinène, a compound found at approx. 1.5% in fresh lemon oil decreased
405 very rapidly at pH 3.0, and lost 85% of its original content during storage. NaC-T-control
406 sample at pH 6.8 showed higher emulsion stability than pH 3.0 due to its high protein
407 solubility (Fig. 5). The use of both conjugated materials to reduce pinene degradation did not

408 modify the degradation trend since the emulsion made with the Maillard derivatives still
409 yielded a very pronounced decline in the order of 90% and 70% at pH 3 and 6.8, respectively.
410 Although combined heating and enzyme treatments did improve the stability of the emulsion
411 compared to glycation alone, however, values obtained were below their respective control
412 samples. The degradation of β-pinene present in the lemon oil over storage period could favor
413 the formation of peroxides. It is known that highly bonded compound such as terpinene and
414 pinene can be oxidized to peroxides and lead to the formation of compounds that produce
415 undesirable off-flavors.[58]

416 **Carvone.** Carvone is often referred as the degradation product of terpenic compounds
417 such as d-limonene under aerobic condition.[59] Therefore, the impact of emulsifier type on
418 limonene oxidation was also followed by monitoring the formation of carvone and terpineol.

419 As seen in Fig. 6, the carvone increase is more pronounced with the two NaC-T-
420 controls (unglycated material) used as protective membrane around the dispersed oil phase of
421 lemon oil at both pH values. On the other hand, sodium caseinate-lactose conjugates reduced
422 the release of carvone probably by slowing down the oxidative degradation of terpenic
423 compounds. Glycation has been reported to enhanced emulsifying activity because covalent
424 linking of polysaccharides rendered proteins more amphiphilic, therefore, more surface active
425 and adsorbable at the O/W interface.[60] Thus, these data suggest that NaC-Lact
426 glycoconjugates were able to inhibit the oxidative deterioration of limonene in oil-in-water
427 emulsions and formation of terpenic compounds. Samples that have been subjected to
428 enzymatic hydrolysis followed by glycation showed higher inhibit of the oxidative
429 deterioration of carvone than their unhydrolyzed counterparts. In addition, at neutral pH (pH
430 6.8), the results indicated higher stability of the emulsion to peroxidation compared to pH 3.0.
431 Schieberle and Grosch [6,7] reported that carvone contributes significantly to the off-flavor of

the peroxidized lemon oil, therefore, the use of glycated sodium caseinate could be a useful approach for preventing the deterioration of lemon oil and extending its storage.

α-Terpineol. α-Terpineol (0.4% in fresh lemon oil) has been recognized as a major objectionable flavor in orange juice and other citrus juices as it imparts a stale, musty and piney odor.[61] Its formation is related to the degradation of limonene and linalool.[4] As shown in Fig. 6, the formation of α-terpineol increased during storage period, particularly at pH 3.0. However, α-terpineol concentration did not change during the storage for pH 6.8 emulsions. This difference related to the pH is relevant to citrus juice, since the pH of lemon juice is generally around 2.8. Under acidic conditions, and depending on the oxygen content, limonene is easily converted during storage, in part, to α-terpineol and carvone, respectively.[62-64] Interestingly, the results clearly indicated that glycation of NaC with lactose exert a major inhibition on the generation of terpineol compared to the control unglycated material at low pH. These results confirm that limonene degradation and carvone and terpineol formation were less in glycated sodium caseinate-stabilized emulsions than in NaC-control-stabilized emulsions. The increase stability of limonene in glycated stabilized emulsions could be attributed to possible conformational and hydrophobicity changes, where the hydrophobic protein being firmly absorbed to the surface of the oil droplet, while the bound hydrophilic polysaccharide is highly solvated by the aqueous phase [9], resulting in higher encapsulation efficiency and stability. Additionally, Maillard reaction products have been reported to exhibit significant antioxidant properties in food, inhibit the oxidative degradation of natural organic compounds.[65-68] Thus, NaC-T-Lact glycoconjugates, as antioxidants, could have contributed to improve the oxidative stability of lemon oil in emulsion systems.

It is also important to note that there was no established stoichiometric correlation between the degradation of limonene and the generation of the degradation products, terpineol

457 and carvone. Both of these by-product compounds can undergo chemical degradation leading
458 to a decrease in their content and the formation of numerous other undesirable off-flavors.

459 ## Conclusion

460 This study led to development of the HS–SPME conditions for the detection and
461 quantification of four oil components (d-limonene, β-pinene, cis-citral and trans-citral), and
462 two degradation products (terpineol and carvone) present in O/W emulsions prepared with
463 NaC and NaC-Lact glycoconjugates. The proposed method is simple to use and could prove
464 useful for the time degradation studies of targeted flavor compounds in beverage emulsions.
465 The compositional changes in a commercial lemon essential oil demonstrated that the use of
466 Maillard conjugates have the potential to improve the protection of the oxygen sensitive
467 flavor oil. The results indicated that glycated sodium caseinate improved the chemical
468 stability of different lemon oil components in O/W emulsions compared to unglycated
469 samples. In addition, pH of the medium had significant effect on the citral and limonene
470 stability. Both compounds degraded slowly at low pH. This might be due to the ability of
471 protein-sugar emulsifier system to form a bulkier polymeric layer and positively charged
472 interfacial membrane that stabilizes the emulsion droplet through steric and electrostatic
473 interactions. In conclusions, sodium caseinate alone was not effective at preventing the acid-
474 promoted degradation reactions but did help protect against oxidation reactions at neutral
475 conditions. Emulsions stabilized with NaC-Lact conjugates was more effective in preventing
476 the degradation of lemon oil in oil-water emulsions as compared to NaC alone; however, the
477 formation of citral and limonene degradation products was higher under acidic NaC-Lact
478 stabilized emulsions. Nevertless, the use of glycated proteins to emulsify citrus oils
479 commonly added to foods and beverages could provide a novel technique to stabilize citral
480 and limonene against oxidative degradation.

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References

1 R. L. Rouseff, and M. Naim, *Citrus flavor stability*. ACS Symposium Series, Flavor Chemistry ISBN13: 9780841236400, 2000.

2 D. Djordjevic, L. Cercaci, J. Alamed, D. J. McClements, and E. A. Decker, *J. Food Sci.,* 2008, **73**, C167–C172.

3 V. R. Bank, D. T. Bailey, and J. T. Van Leersum, *Storage stable, citrus-flavored compositions comprising plant extracts*, *US Pat*., 6638555. 2003.

4 E. Haleva-Toledo, M. Naim, U. Zehavi, and R. L. Rouseff, *J. Food Sci.,*1999, **64**, 838–841.

5 P. Oliveira, M. L. Rojas-Cervantes, A. M. Ramos, I. M. Fonseca, A. M. Botelho do Rego, and J. Vital, *Catalysis Today*, 2006, **118**, 307-314.

6 P. Schieberle, and W. Grosch, *J. Agric. Food Chem*., 1988, **36**, 797-800.

7 P. Schieberle, and W. Grosch, *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 1989, **189**, 26-31.

8 M. P. Ennis, and D. M. Mulvihill, In *Handbook of Hydrocolloids*, G.O. Phillips and P.A. Williams, Woodhead Publishing Limited, Cambridge, 2000, 189–217.

9 J. O'Regan, and D. M. Mulvihill, *Food Chem.,* 2009, **115**, 1257–1267.

10 C. M. Oliver, L. D. Melton, and R. A. Stanley, *J. Sci. Food Agric.,* 2006, **86**, 732–740.

11 C. M. Oliver, M. A. Augustin, and L. Sanguansri, *Australian J. Dairy Technol*., 2009, **64**, 80-83.

12 U. Einhorn-Stoll, M. Ulbrich, S. Sever, H. Kunzek, *Food Hydrocolloids*, 2005, **19**, 329-340.

13 A. Fechner, A. Knoth, I. Scherze, and G. Muschiolik, *Food Hydrocolloids*, 2007, **21**, 943–952.

14 M. Corzo-Martínez, A. C. Soria, M. Villamiel, A. Olano, F. M. Harte, and F. J. Moreno, *J. Dairy Sci.,*2011, **94**, 51–58.

15 T. J. Wooster, and M. A. Augustin, *J. Colloid Interface Sci*., 2006, **303**, 564-572.

16 J. M. Chobert, J. C. Gaudin, M. Dalgalarrondo, and T. Haertlé, *Biotechn. Advances*, 2006, **24**, 629–632.

17 J. O'Regan, and D. M. Mulvihill, *Food Res. Inter.*, 2010, **43**, 224-231.

18 M. A. Augustin, L. Sanguansri, and O. Bode, *J. Food Sci*., 2006, **71**, E25-E32.

19 U. Lesmes, and D. J. McClements, *Food Hydrocolloids*, 2012, **26**, 221-230.

20 J. O'Regan, and D. M. Mulvihill, *Food Chem.,* 2010, **119**, 182-190.

21 S. S. Marine, and J. Clemons, *J. Chromat. Sci.,* 2003, **41**, 31–35.

22 D. Djordjevic, L. Cercaci, J. Alamed, D. J. McClements, and E. A. Decker, *J. Agric. Food Chem.,* 2007. **55**, 3585–3591.

23 D. Djordjevic, L. Cercaci, J. Alamed, D. J. McClements, and E. A. Decker, *Food Chem.,* 2008, **106**, 698–705.

24 A. Achouri, J. I. Boye, and Y. Zamani, *Food Chem.,* 2006, **99**, 759–766.

25 J. Januszkiewicz, H. Sabik, S. Azarnia, and B. Lee, *J. Chromatography A,* 2008, **1195**, 16–24.

26 F. C. Church, H. E. Swaisgood, D. H. Porter, and G. L. Catignani, *J. Dairy Sci.,*1983, **66**, 1219-1227.

27 L. Greenspan, *J. Res. National Bureau of Standard*s, 1977, **81A**, 89-96.

28 A. A. Betschart. *J. Food Sci*., 1974, **39**, 1110–1115.

29 M. M. Bradford, *Anal. Biochem*., 1976, **72**, 248–254.

30 K. N. Pearce, and J. E. Kinsella, *J. Agric. Food Chem*., 1978, **26**,716–723.

31 W. Chantrapornchai, F. M. Clydesdale, and D. J. Mc Clement, *J. Agric. Food Chem*., 1998, **46**, 2914–2920.

32 B. Cammerer, and L. W. Kroh, *Food Chem.*, 1996, **57**, 217-221.

33 F. J. Morales, and S. Jimenez-Perez, *Food Chem.,* 2001, **72**, 119-125.

34 O. Pinho, C. Pérès, and I. M. P. L. V. O. Ferreira, *J. Chromatography A,* 2003, **1011**, 1–9.

35 J. Pawliszyn, *Solid Phase Microextraction: Theory and Practice*. Wiley-VCH: New York, NY, USA, 1997.

36 R. T. Marsili, *J. Agric. Food Chem.,* 1999, **47**, 648–654.

37 C. Pérès, C. Viallon, and J. L. Berdagué, *Anal. Chem.,* 2001, **73**, 1030–1036.

38 H. Kataoka, H. L. Lord, and J. Pawliszyn, *J. Chromatography A,* 2000, **880**, 35–62.

39 H. Prosen, and L. Zupančič-Kralj, *Trends in Analytical Chemistry,* 1999, **18**, 272–282.

40 B. Lorrain, J. Ballester, T. Thomas-Danguin, J. Blanquet, J. M. Meunier, and Y. Le Fur, *J. Agric. Food Chem.,* 2006, **54**, 3973–3981.

41 [FDA] Food and Drug Administration. at: http://fda.gov./cder/guidance/index.htm. 2001.

42 S. Katayama, J. Shima, and H. Saeki, *J. Agric. Food Chem*., 2002, **50**, 4327-4332.

43 R. Sato, T. Sawabe, H. Kishimura, K. Hayashi, and H. Saeki, *J. Agric. Food Chem*., 2000, **48**, 17-21.

44 M. Tanabe, and H. Saeki, *J. Agric. Food Chem*., 2001, **49**, 3403-3407.

45 S. Y. Lee, C. V. Morr, and E. Y. W. Ha, *J. Food Sci.*, 1992, **57**, 1210-1213.

46 R. Groubet, J.-M Chobert, and T. Haertle, *Sciences des Aliments*, 1999, **19**, 423–438.

47 R. Shepherd, A. Robertson, and D. Ofman, *Food Hydrocolloids*, 2000, **14**, 281–286.

48 T. P. A. Devasagayam, J. C. Tilak, K. K. Boloor, K. S. Sane, S. S. Ghaskadbi, and R. D. Lele, *J. Assoc. Phys. India*, 2004, **52**, 794-804.

49 Y. G. Guan, H. Lin, Z. Han, J. Wang, S. J. Yu, X. A. Zeng, and et al. *Food Chem.*, 2010, **123**, 275–280.

50 M. S. Rao, S. P. Chawla, R. Chander, and A. Sharma, *Carbohydrate Polymers*, 2011, **83**, 714–719.

51 H. Y Wang, H. Qian, and W. R. Yao, *Food Chem.*, 2011, **128**, 573–584.

52 Y. Joubrana, A. Mackie, and U. Lesmes, *Food Chem.*, 2013, **141**, 3796–3802.

53 A. C. Bertolini, A. C. Siani, and C. R. F. Grosso, *J. Agric. Food Chem.,* 2001, **49**, 780–785.

54 S. Anandaraman, and G. A. Reineccius, *Food Technology,* 1986, **40**, 88–93.

55 F. Jahaniaval, Y. Kakuda, V. Abraham, and M. F. Marcone, *Food Res. International*, 2000, **33**, 637-647.

56 J. Ventureira, E.N. Martinez, M.C. Anon, *Food Hydrocolloids*, 2010, **24**, 551–559.

57 H. Nguyen, E. M. Campi, W. R. Jackson, and A. F. Patti, *Food Chem.*, 2009, **112**, 388–393.

58 D. Wabner, *Intern. J. Aromatherapy,* 2002, **12**, 216–218.

59 J. Pan, H. Wang, H. Xie, Y. Yang, and Q. Zeng, *J. Food Proc. Engin.,* 2011, **34**, 728–745.

60 X. Guo, and Y. L. Xiong, *LWT - Food Sci. Technol.,* 2013, **51**, 397-404.

61 S. Elss, S. Kleinhenz, and P. Schreier, *LWT-Food Sci., Technol.,* 2007, **40**, 1826–1831.

62 J.M. Tatum, S. Nagy, and R. E. Berry, *J. Food Sci.*, 1975, **40**, 707–709.

63 P. Durr, U. Schobinger, and R. Maldvoger, *Lebensmitt. Verpackungs,* 1981, **20**, 91–93.

64 M.A. Petersen, D. Tonder, and L. Poll, *Food Quality and Preference*, 1998, **9**, 43–51.

65 B. J. Mc Gookin, and M. A. Augustin, *J. Dairy Research*, 1991, **58**, 313–320.

66 M. Namiki, *Crit. Rev. Food Sci. Nutr*., 1990, **19**, 273–276.

67 S. Drusch, S. Berg, M. Scampicchio, Y. Serfert, V. Somoza, S. Mannino, K. Schwarz, *Food hydrocolloids*, 2009, **23**, 942–948.

68. X. Huang, Z. Tu, H. Xiao, H. Wang, L. Zhang, Y. Hu, Q. Zhang, P. Niu, *Food Res. International*, 2012, **48**, 866–872.

Figures Captions

Fig. 1 Schematic diagram of (A) citral and (B) d-limonene degradation pathway

Fig. 2 Mass spectrometer detector response (peak area) of extracted volatile compounds using the three different SPME fibers.

Fig. 3 Time dependent degradation of d-limonene in lemon oil emulsions stabilized with NaC-T-Lactose glycoconjugates at pH 3.0 and 6.8. Data markers represent average (n=3) ± standard deviations.

Fig. 4 Time dependent degradation of cis- and trans-citral in lemon oil emulsions stabilized with NaC-T-Lactose glycoconjugates at pH 3.0 and 6.8. Data markers represent average (n=3) ± standard deviations.

Fig. 5 Time dependent degradation of β-pinene in emulsions stabilized with NaC-T-Lactose glycoconjugates at pH 3.0 and 6.8. Data markers represent average (n=3) ± standard deviations.

Fig. 6 Time dependent generation of carvone and α-terpineol in emulsions stabilized with NaC-T-Lacose glycoconjugates at pH 3.0 and 6.8. Data markers represent average (n=3) ± standard deviations.

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657 **Table 1** Calibration curve characteristics for CAR/PDMS fibre (n = 3)

Component	Retention time (min)	Repeatability (RSD)	R^2	Detection limit (ng/g oil)
D-Limonene	12.00	14.8	0.972	0.08
Cis-citral	14.45	7.1	0.997	0.02
Trans-citral	14.67	7.5	0.997	0.02
β-Pinene	10.94	11.5	0.988	0.92
Carvone	14.55	6.3	0.999	0.05
Terpineol	14.09	7.2	0.996	0.05

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680 **Table 2** Functional properties and antioxidant capacity of lactose glycated sodium

681 caseinate

Sample	Solubility (%)		E.A.I (m^2g^{-1})	%$DPPH_{rem}$
	pH 3	pH 6.8		
NaC-T-control	61.3±1.3a	94.5±1.9a	13.8±1.7a	99.2±0.4a
NaC-T+Lactose	38.9±2.4b	59.9±3.1c	12.6±0.7a	97.3±0.7b
NaC-T/E+Lactose	58.4±2.4a	88.3±1.3b	13.7±0.4a	96.5±0.2b

682 Different letters within the same column indicate statistically significant differences among samples ($P<0.05$).
683 Data are means±standard deviation of triplicates.
684 NaC-T: Sodium caseinate heated at 80 °C for 30 min.
685 E.A.I: Emulsion activity index.
686 %$DPPH_{rem}$: express the percentage of remaining DPPH-radical scavenging activity.
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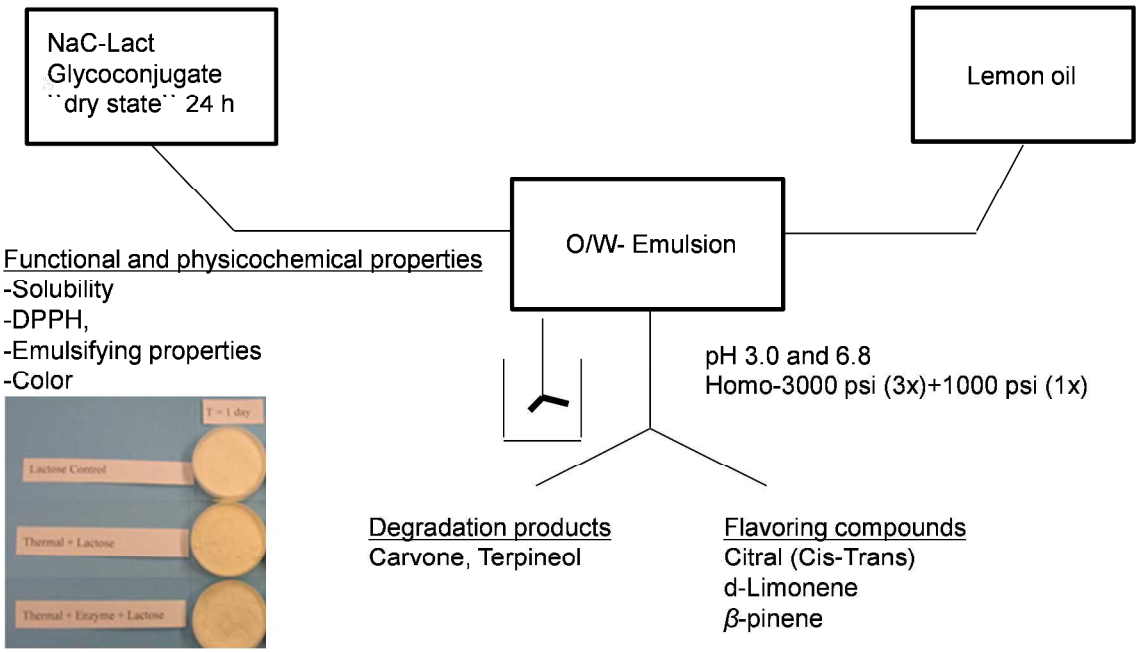
Table 3 Hunterlab colour values of control and lactose glycated sodium caseinate

Sample	*L*	*a*	*b*	ΔE (color)
NaC-T-control	91.67	- 1.36	10.82	0.81b
NaC-T+Lactose	91.82	- 1.08	10.97	1.07b
NaC-T/E+Lactose	92.81	- 0.85	11.01	1.82a

Different letters within the same column indicate statistically significant differences among samples ($P<0.05$).
Data are means±standard deviation of triplicates.
ΔE: color difference; values were calculated using the following formula: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$, where ΔL, Δa and Δb are the differences in the specified tristimulus coordinate between the sample and sodium caseinate used as control. The '*L*' scale denotes lightness-to-darkness in 100–0 units. The '*a*' scale represents redness (+a) vs. greenness (-a) and the '*b*' scale represents yellowness (+b) vs. blueness (-b).



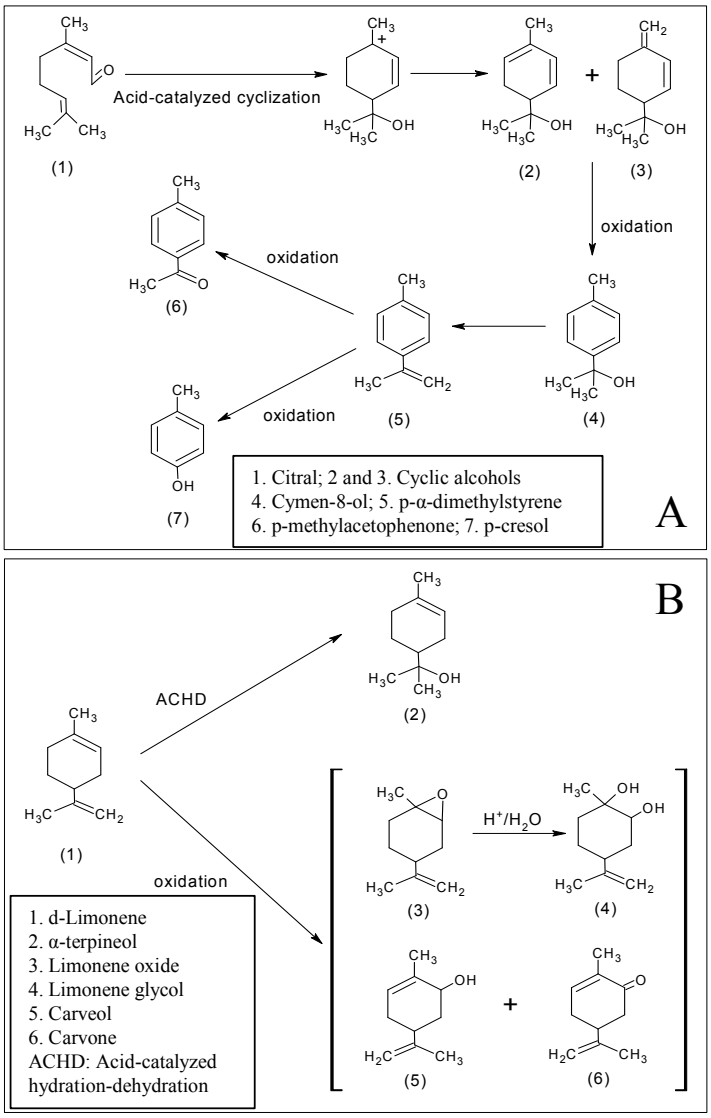


Fig. 1

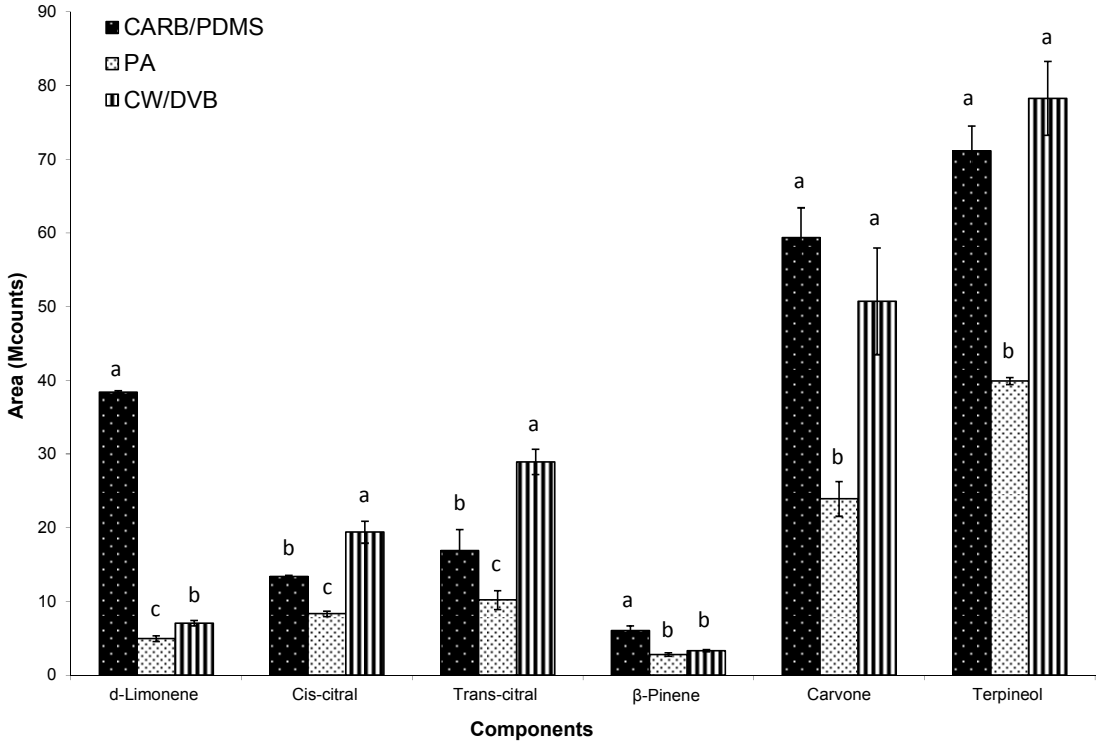


Fig. 2

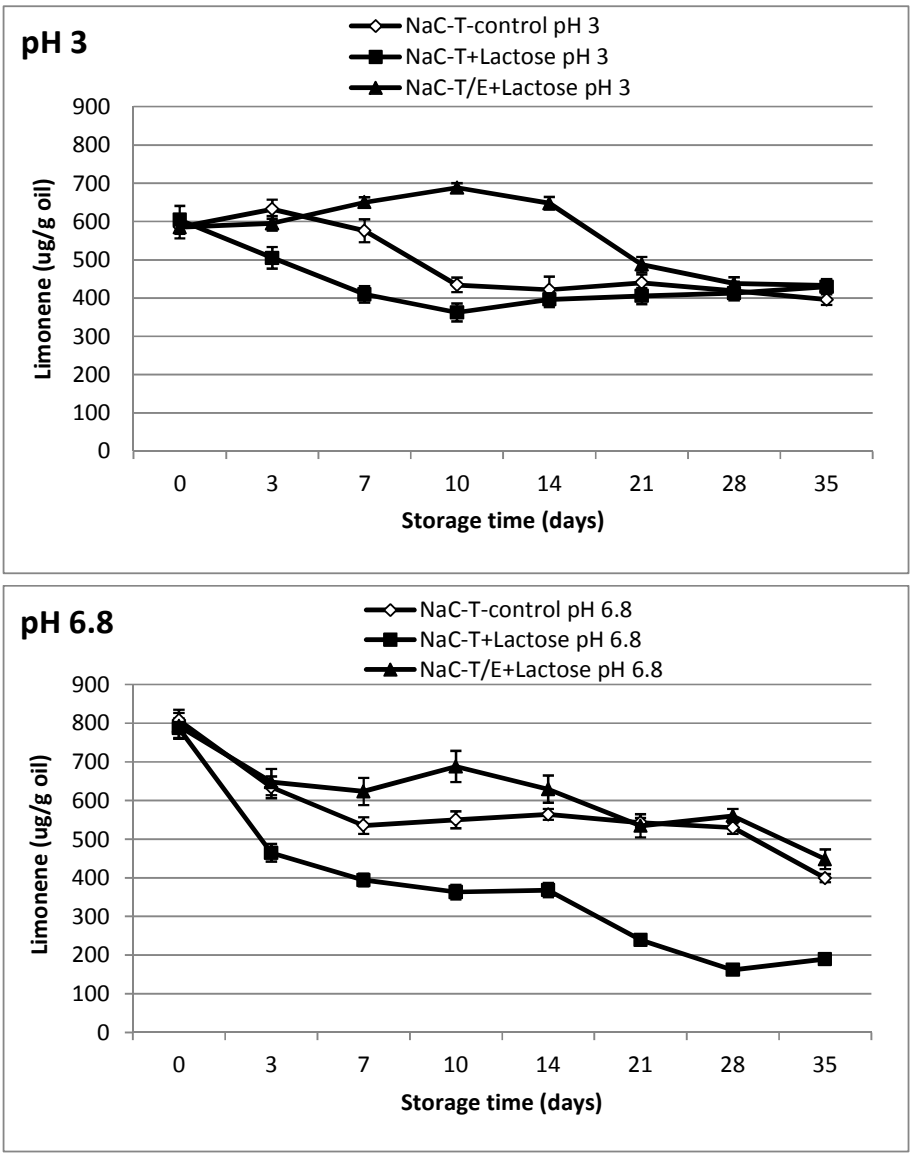


Fig. 3

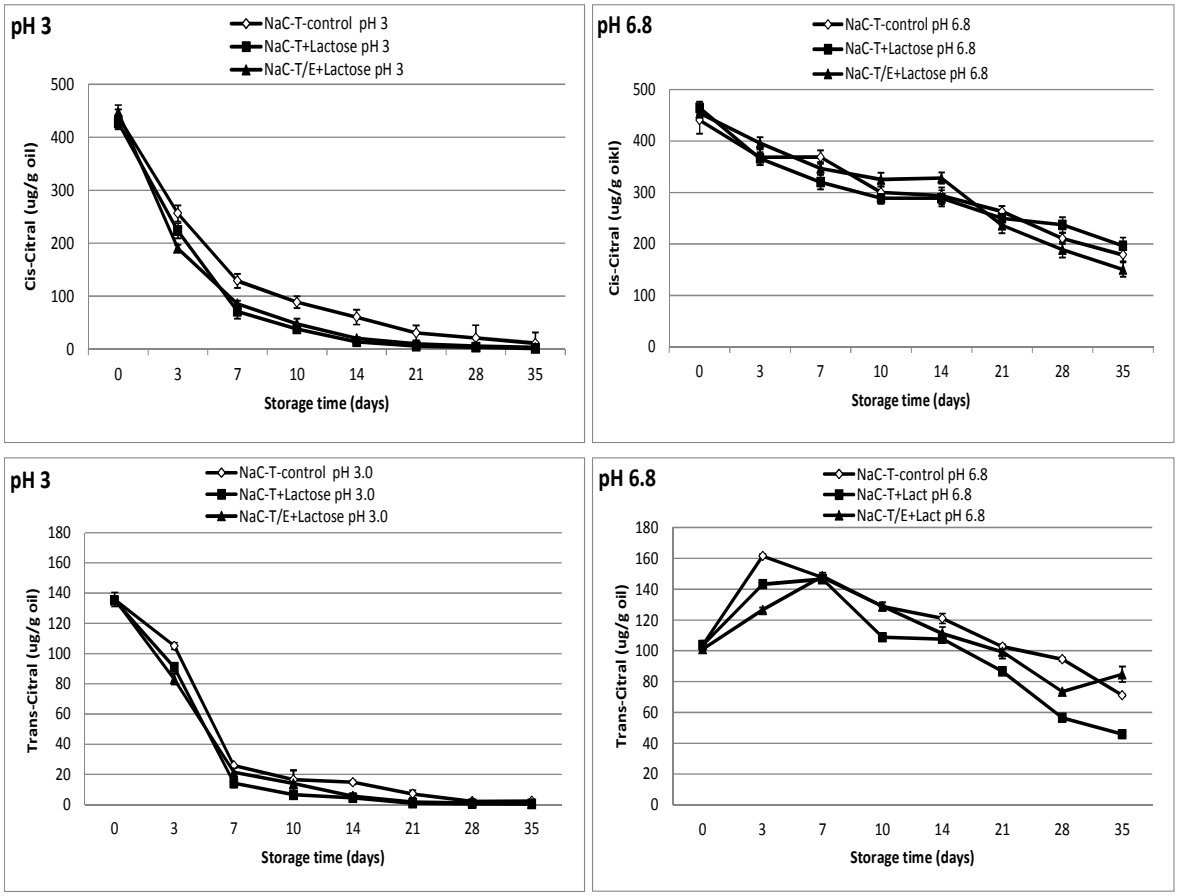


Fig. 4

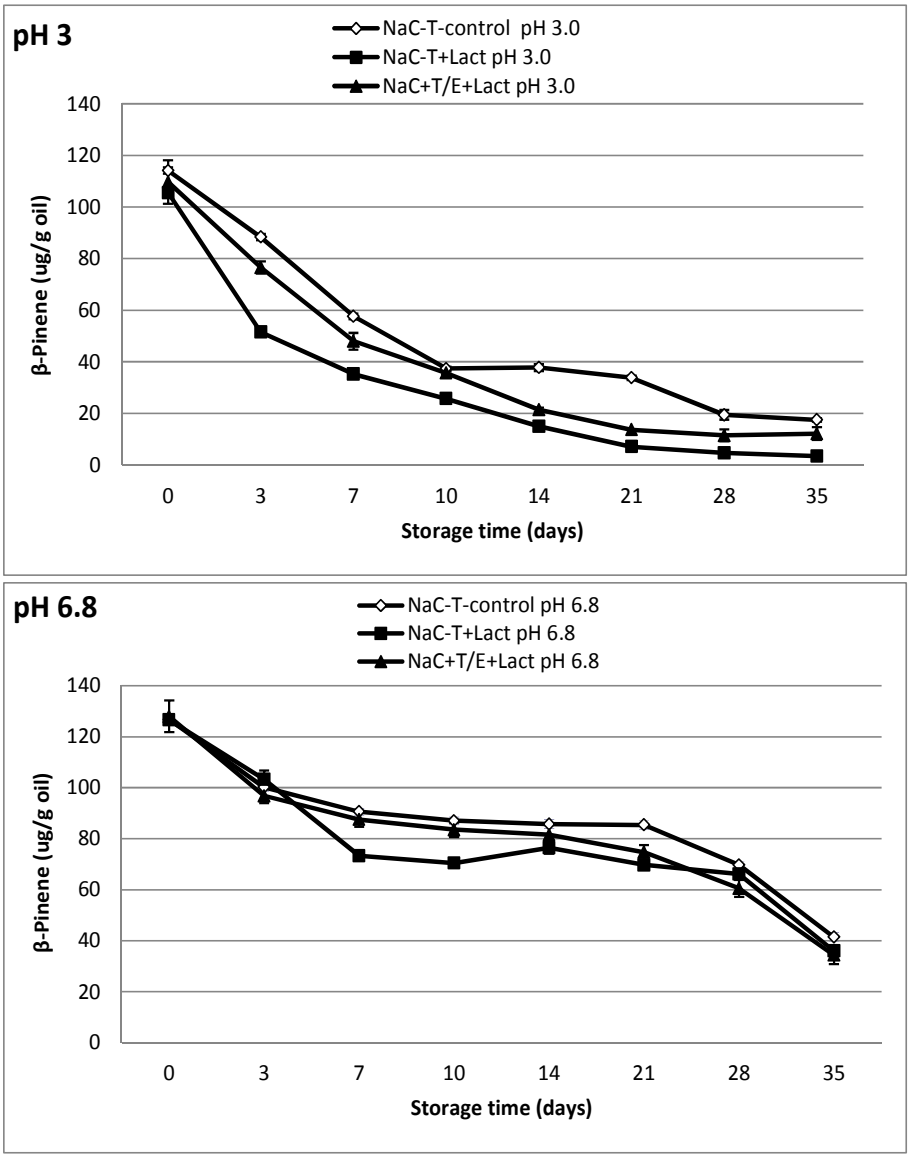


Fig. 5

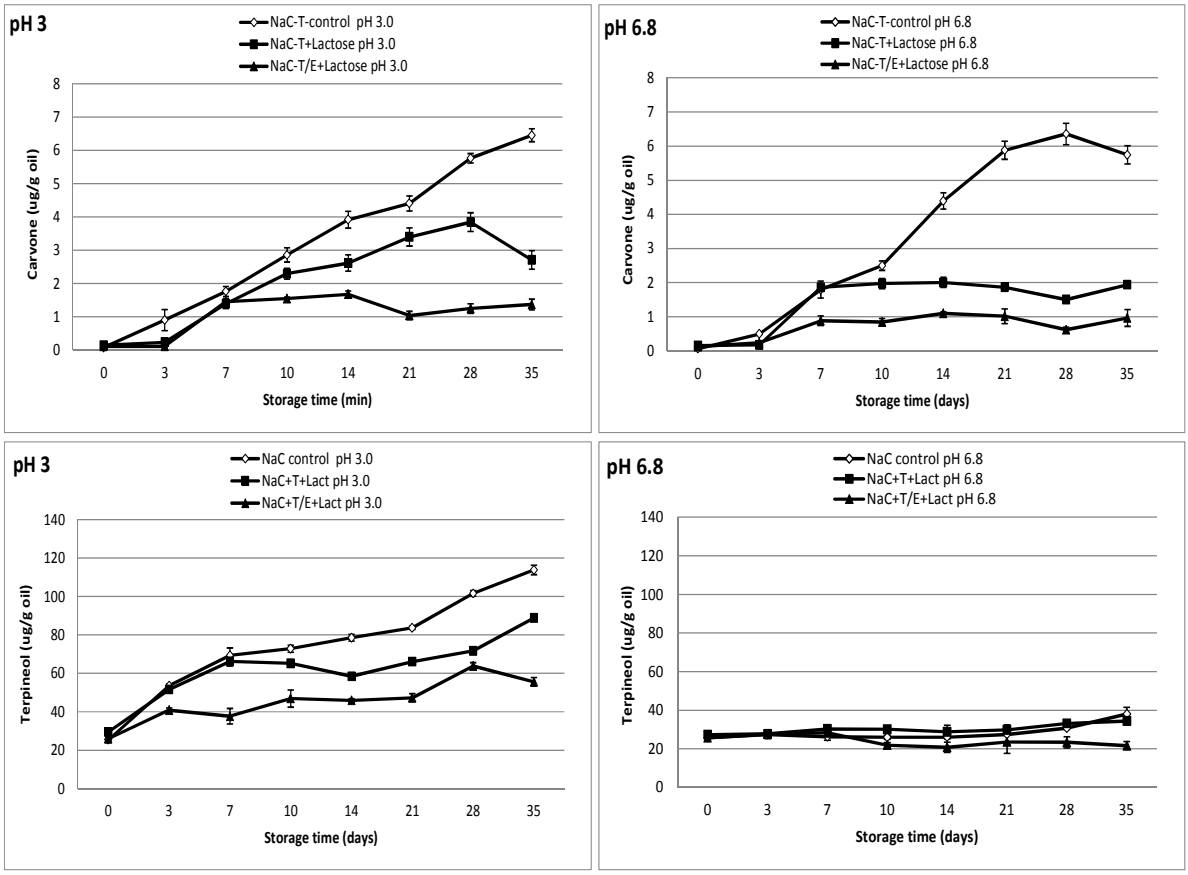


Fig. 6