

1 Running head: **Spray-drying of citral in sugar matrices**

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4 **Spray drying encapsulation of citral in sucrose or trehalose matrices:**

5 **Physicochemical and sensory characteristics**

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24

25 **Abstract**

26

27 The potential of the disaccharide trehalose as carrier for producing spray-dried
28 citral was examined. Some physical and sensory characteristics of citral encapsulated in
29 matrices containing either trehalose or sucrose and maltodextrin (MD) were analyzed.
30 They included water sorption, glass transition temperatures (T_g), NMR relaxation, and
31 citral retention during spray drying.

32 The glassy state at room temperature (25 °C) was maintained as far as 33 % RH
33 for the spray dried trehalose formulation and 43 % RH for trehalose-MD; however for
34 sucrose-MD and for sucrose formulations, the glassy state was limited to relative
35 humidities lower than 33 % and 22 % RH, respectively. Sensory evaluation and gas
36 chromatographic analysis indicated that citral retention after spray drying was similar for
37 matrices containing either trehalose or sucrose. However, trehalose formulations had
38 improved physical stability as compared to sucrose.

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40 **Keywords:** citral; trehalose; sucrose; encapsulation; glass transition temperature; NMR;
41 spray drying

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44 **1. Introduction**

45 Microencapsulation of hydrophobic flavors is of great importance in the flavoring
46 and food industries, since solid or liquid microencapsulated food flavors exhibit good
47 chemical stability and a controlled release. Citral (3,7-dimethylocta-2,6-dienal) is an
48 important flavor component that is present in essential oils of lemon grass, orange, and

49 lemon. It is widely used as flavoring or fragrance agents in foods and cosmetics for its
50 strong lemon odor. It is a mixture of two terpenoids (geranial and neral), which are
51 double bond isomers. Geranial has a strong lemon odor and neral's odor is less intense,
52 but sweeter. Citral is highly susceptible to oxidation and volatilizes rapidly leading to the
53 loss of aroma and formation of off-flavors (Reineccius, 2006). To overcome these
54 problems, microencapsulation of citral in protective matrices is frequently employed
55 (Bhandari *et al.*, 1992; Loksuwan, 2009). Spray-drying is used to produce flavor
56 powders in a short time due to its low cost and easiness (Gharsallaoui *et al.*, 2007).

57 The choice of a wall material for microencapsulation by spray-drying is very
58 important for encapsulation efficiency (i.e. % flavour retention during drying) and
59 microcapsule stability during storage. A single encapsulating agent cannot possess all
60 ideal properties; therefore carrier systems are usually formulated by combining a
61 number of water-soluble components (Gharsallaoui *et al.*, 2007). In this sense, one
62 option is to use mixtures containing modified starches, which present good
63 emulsification and lipid-droplet-stabilization properties (Porzio, 2007), and polymers like
64 maltodextrin (MD), which provide a low cost, bland component, and also offers good
65 oxidative stability to encapsulated oil (Kenyon, 1995).

66 It is well known that the type of carrier also governs flavor retention during the
67 spray drying process (Chirife & Karel, 1973; Menting *et al.*, 1970; Thijssen, 1971); and
68 for this reason disaccharides (i.e. sucrose) are sometimes included with MD in
69 commercial formulations to improve retention characteristics. Sucrose and particularly
70 glucose, were reported not to be suitable for spray-drying flavors because they may
71 adhere to the surface of the spray-drier and clogging the nozzle (Bayram *et al.*, 2005).
72 On spite of these problems, sucrose is sometimes used mixed with MD and other carrier

73 components because it contributes to flavour retention (and flavour profile) of the
74 encapsulated material. However, the presence of sucrose in the carrier formulation
75 affected storage stability in a negative way, because it reduced the glass transition
76 temperature dramatically, as recently shown by Busso Casati *et al.*, (2007) for some
77 commercial strawberry and orange spray-dried powder flavors.

78 An important concept in the material science of amorphous carbohydrates is the
79 glass transition, which is used to predict the processing and physical stability of
80 carbohydrate-based food products. The glass transition temperature (T_g), which
81 separates the supercooled from the glassy state (Ablett *et al.*, 1993; Levine & Slade,
82 1986; Slade & Levine, 1991; Roos, 1995) is indicative of the degree of molecular
83 mobility of the amorphous matrix. In the supercooled state, translational and rotational
84 motion of the matrix molecules is possible, but in the glassy state large-scale molecular
85 motion is inhibited (Angell, 1988). Mobility of small molecules within the matrix is
86 possible in the glassy state, for instance the mobility of water, gases, and small organic
87 molecules (Tromp *et al.*, 1997; Schoonman *et al.*, 2002; Ubbink & Reineccius, 2002).
88 NMR relaxation has been proposed as a valuable method to understand the relationship
89 between the molecular mobility of water and sugars and the moisture content and
90 temperature above and below the glass transition temperature (Hills *et al.*, 2001).

91 Trehalose is a non-reducing disaccharide which consists of two glucose
92 molecules linked in a 1,1-position by an α -glycosidic bond. The glass transition
93 temperature for trehalose is much higher than that of sucrose which would contribute to
94 the physical stability of spray-dried matrices containing trehalose instead of sucrose.
95 Komes *et al.* (2003, 2005) observed that the addition of trehalose previous to

96 dehydration of strawberry and apricot purees resulted in the lowest loss of total aroma,
97 as well as of individual fruit volatiles when compared with sucrose.

98 The potential of a carrier to produce spray-dried flavors must be evaluated from
99 both the ability to entrap the volatiles during the drying process as well as the protection
100 afforded during storage. It is the objective of the present work to compare some physical
101 and sensorial characteristics of spray-dried citral formulations containing either sucrose
102 or trehalose, mainly from the point of view of encapsulation efficiency during spray-
103 drying. For this purpose a model system using citral as volatile aroma is studied.

104

105 **2. Materials and methods**

106 2.1. Materials

107 Sucrose (S) and trehalose dihydrate (T) of analytical grade, were purchased from
108 Merck (Germany) and Cargill Inc. (Wayzata, MN, USA), respectively, and Maltodextrin
109 (MD) DE 12 was from Givaudan S.A. (Argentina). The modified commercial starch used
110 as emulsifier was Capsul (Gelfix, Argentina). The dispersed phase was constituted by
111 the essential oil citral Extra Fino, kindly given by Saporiti Sabores (Argentina). Deionized
112 water (Milli Q) was used in all cases.

113

114 2.2. Model systems preparation

115 Pre-emulsions were formed by adding 7.8 % citral oil to premixtures formed by a)
116 37.5 % water, 4.7 % starch and either 50 % sucrose (S) or trehalose (T), or b), a
117 mixture (1:1) of 25 % MD plus 25 % S (SM) or 25 % MD plus 25 % T, (TM).

118 200 ml of each pre-emulsion were obtained by mixing the ingredients in a Griffin &
119 George (Loughborough, UK) stirrer. This operation was carried out for 2 min, at 25 °C

120 and 750 rpm. Emulsions formation was completed by mixing the pre-emulsion with a
121 high speed blender, Sorvall OMNI MIXER 17106 -OMNI Corporation International,
122 Waterbury (CT, USA), operating at 16000 rpm for 10 min. Temperature increase was
123 avoided by keeping the sample into an ice bath during stirring. The pH values were 3.49
124 ± 0.01 for T and S emulsions and 4.04 ± 0.01 for those containing MD.

125

126 *2.2.1. Emulsions Spray-drying*

127 The emulsions were spray-dried using a laboratory scale device, Mini Spray Dryer
128 Büchi B290 (Flawil, Switzerland). The operational conditions of the drying process were:
129 inlet air temperature of 175 ± 3 °C, outlet air temperature 83 ± 3 °C, **flow rate 8 mL/min,**
130 **air pressure 3.2 bar, nozzle diameter 1.5 mm.** The obtained powders were collected into
131 sealed PVDC bags and then stored at about 18 °C.

132

133 *2.2.2. Humidification*

134 Approximately 4 g of the spray-dried powders were loaded into 5 ml vials and
135 immediately transferred into evacuated desiccators which were kept for 14 days at 23 °C
136 over saturated salt solutions that provided constant relative humidities (RH) of 11 %
137 (LiCl), 22 % (CH₃COONa), 33 % (MgCl₂), and 43 % (K₂CO₃) (Greenspan, 1977).

138

139 *2.3. Methods*

140

141 *2.3.1. Water activity*

142 Water activity was measured using an electronic dew-point water activity meter
143 Aqualab Series 3 (Decagon Devices, Pullman, Washington, USA). The measurements

144 were done at room temperature. For each determination three replicates were
145 measured. After spray-drying the a_w values of the powders ranged between 0.098 and
146 0.183 with standard deviations lower than 0.005.

147

148 *2.3.2. Water content*

149 Karl Fisher (KF) titration was carried out at 25 ± 1 °C with a Karl Fischer titrator
150 DL 31 from Mettler-Toledo, applying the one-component technique with Hydranal Titrant
151 Composite 5 from Riedel-de Haën, Germany. Pure methanol or a methanol:formamide
152 mixture 95 (1:1) were used as solvent and they were purchased from Merck (Darmstadt,
153 Germany). Sample sizes were approximately 100 mg and standard deviation was
154 calculated from six replicate measurements.

155

156 *2.3.3. Thermal transitions*

157 Glass transitions were determined by differential scanning calorimetry (DSC;
158 onset values) using a DSC 822e Mettler Toledo calorimeter (Schwerzenbach,
159 Switzerland). The instrument was calibrated with water (0.0 °C), indium (156.6 °C), lead
160 (327.5 °C) and zinc (419.6°C). All measurements were performed at a heating rate of 10
161 °C/min., and hermetically sealed 40 μ l medium pressure pans were used (an empty pan
162 served as reference). Thermograms were evaluated using Mettler Star^e program. An
163 average value of at least two replicates was reported. **The confidence interval for the
164 glass transition temperature values was 2 °C.**

165

166 *2.3.4. Molecular mobility*

167 A Bruker PC 120 Minispec pulsed proton nuclear magnetic resonance ($^1\text{H-NMR}$)
168 instrument, with a 0.47 T magnetic field operating at resonance frequency of 20 MHz,
169 was used for measurements. Equilibrated samples were removed from the desiccators,
170 placed into 10 mm diameter glass tubes and returned to the desiccators for 24 h prior to
171 analysis.

172 The spin-spin relaxation time (T_2) associated to the fast relaxing protons (related to the
173 solid matrix and to water interacting tightly with solids) was measured using a free
174 induction decay analysis (FID) after a single 90° pulse. The decay envelopes (protons
175 signal intensity (I) versus experimental time (t)) were fitted to mono-exponential behavior
176 with the following equation:

$$177 \quad I = A \exp (-t/T_2)$$

178 where T_2 corresponds to the relaxation time of protons in the polymeric chains of the
179 sample and of tightly bound water, and A is a constant. Since no 180° refocus pulse was
180 used in the experiments, the spin-spin relaxation time constants are apparent relaxation
181 time constants, i.e. T_2^* . However for solid samples (like ours), we can consider that the
182 intrinsic T_2 is very close to the T_2^* as reported previously by Fullerton and Cameron
183 (1988). Therefore, T_2 was used for convenience.

184

185 *2.3.5. Gas chromatography analyses*

186 - Citral extraction: 0.2000 g of spray-dried powder were placed in a 10 mL glass vial and
187 3 mL of water were added. Samples were mixed for 3 minutes in a vortex mixer and
188 sonicated at 35°C for 30 minutes. 2 mL pure hexane were added to the suspensions and
189 samples were stirred for 3 minutes and centrifuged at 3500 rpm for 5 minutes. The oily
190 phase was collected and this last procedure was repeated 4 times. 1.0 mL of linalool

191 (internal standard) was added to the final oily phase and the final volume (25 mL) was
192 completed with hexane.

193 - GC analyses: Citral components were separated by GC method using a Varian device
194 with a flame ionization detector (FID), with a DB5 capillary column (60 m x 250 μm).
195 Temperature of the column was programmed from 75 to 210 $^{\circ}\text{C}$, rise rate 3 $^{\circ}\text{C}/\text{min}$,
196 injector temperature 250 $^{\circ}\text{C}$, and detector temperature 260 $^{\circ}\text{C}$. Two replicates for each
197 sample were used. The content of citral components (neral and geranial) was calculated
198 by the internal standard method.

199

200 2.3.6. *Sensory evaluation*

201 - Paired comparison test

202 Citral **sensory differences** in spray-dried sucrose and trehalose matrices was estimated
203 from sensory evaluation of citral aroma performed in the spray-dried samples, using a
204 forced-choice Paired Comparison (**2-AFC test**; ASTM 1977). A panel of 30 untrained
205 assessors (7 men, 23 women; 21–23 years old; undergraduate students of food
206 science/engineering from Facultad de Ciencias Agrarias, Pontificia Universidad Católica,
207 Argentina) evaluated the four samples (sucrose, trehalose and their mixtures with
208 maltodextrin). Each assessor received two samples (15 mL) presented in three-digit
209 coded sealed glass flasks (35 mL capacity, 3 cm diameter opening) and they had to
210 choose the more aromatic one. Before evaluation, encapsulated citral samples were
211 diluted to 0.05% (mg/100ml) in water, and several dilutions of this initial concentration
212 (0.05%, named **$C_0=6.25\text{mg citral}/100\text{mL solution}$**) were tested in order to find the
213 adequate concentration to work. This dilution was 1/20 of C_0 , which was put in glass
214 flasks and allowed to equilibrate (**4 hrs at 22 ± 2 $^{\circ}\text{C}$**) between liquid and gas phases.

215 Testing was performed in individual booths at a temperature of 22 ± 2 °C and
216 illuminated with white light (6500 K). The significance level of the paired comparison test
217 was calculated by binomial test ($\alpha=0.05$; 2-tailed; critical value=21) based on the number
218 of more aromatic samples.

219

220 - Rating scale method

221 Aromatic intensity of encapsulated citral samples was studied by rating scale method
222 using a 15-cm unstructured scale (ASTM 1977). A panel of 12 assessors (all women;
223 21–23 years old; undergraduate students of food science/engineering from Facultad de
224 Ciencias Agrarias, Pontificia Universidad Católica, Argentina) were trained (two
225 sessions, half hour each) to evaluate aroma intensity of citral solutions which were
226 prepared with dilutions of emulsions (0.04, 0.11, 0.33 and 0.98 mg/100 ml) using the
227 scale. The reference point was the dilution 0.33 mg/100ml, which had a value of 8 on
228 the 15-cm unstructured line scale. The aroma intensity of powder solutions (1/20 of C_0 ,
229 the same dilution used in Paired comparison test) was evaluated in triplicate (four
230 sessions). A calibration curve of citral solutions was made and the aroma intensity of
231 encapsulated citral powders was calculated from this curve.

232 Each assessor tested one powder solution and the four citral concentrations per
233 session, with an interval of 10 min among replications. Evaluation was performed in the
234 same conditions that Paired comparison test.

235

236 Statistical Analysis

237 Analysis of variance (ANOVA, one way) was carried out to assess the citral
238 intensities which are significantly different among powder solutions (SPSS v. 13.0).

239 Multiple means comparisons were carried out by Student–Neuman–Keuls (SNK) test at
240 $P < 0.05$.

241 Citral perceived (% w/v) was estimated considering that spray-dried powders at
242 0.05 % dilution had a theoretical concentration of 6.25 mg citral/100 ml.

243

244 **3. Results and discussion**

245

246 Water sorption, glass transition temperature (T_g , onset) and 1H relaxation times
247 (T_2) were determined for the different spray-dried formulations kept at relative humidities
248 (RH) between 11 and 43 %. This RH range was selected because dehydrated powders,
249 (like present samples), are usually kept at low moisture conditions. Water sorption data
250 obtained after 14 days of humidification at 23 °C are shown in **Table 2**; these values
251 should be referred as “pseudo equilibrium” since for amorphous sugars complete
252 equilibration may take longer times (Makower & Dye, 1956; Yu *et al.*, 2008). As
253 expected, water content increased with increasing RH for all systems. The water content
254 of the model systems containing sucrose (without MD) were similar to those obtained by
255 other authors for pure amorphous sucrose (Aguilera, 2003). Yu *et al.*, (2008) compared
256 pseudo-sorption isotherms for amorphous sucrose from several literature sources,
257 showing a relatively good agreement with present data.

258 Makower and Dye (1956) showed that pure amorphous sucrose crystallizes at
259 room temperature when exposed to 30 % RH. They observed that sucrose
260 crystallization was a time-dependent phenomenon and took more than 12 days storage
261 to start crystallization which was completed after about 22 days. Present sucrose
262 system (**Table 2**) did not show visual evidence of crystallization after 14 days at 33 %

263 RH; this may be attributed to the time-dependence of sucrose crystallization, as noted
264 by Makower and Dye, 1956. Iglesias and Chirife (1978) reported that several polymers
265 delayed sucrose crystallization in amorphous systems and Roos and Karel (1991)
266 reported the inhibition of sucrose crystallization in the presence of starch or corn syrup
267 polymers. The presence of modified starch (and to some extent citral), might explain the
268 delayed sucrose crystallization observed in present work. The trehalose system (without
269 MD) (**Table 2**) presented lower water contents than those reported for pure trehalose
270 (Iglesias *et al.*, 1997) but this difference is likely due to the presence of citral (non-
271 hygroscopic) and modified starch in the formulation which could affect the water sorption
272 behavior. Crystallization was not observed in the trehalose systems and this is in
273 agreement with literature results (Schebor *et al.*, 2010).

274 **Figure 1** shows typical DSC thermograms showing the glass transition
275 temperature (T_g) for the different systems at 11 % RH (a), and T_g values as a function of
276 water content (b). **The shapes of the thermal profiles are characteristic of different**
277 **carbohydrates; sugars samples showed sharp glass transitions while the admixture of**
278 **MD caused a broadening of the glass transition, as reported (referencias).** Clear glass
279 transitions (**Figure 1a**) were apparent for all systems; this was also observed at the
280 other RHs analyzed (not shown). The T_g values of the systems containing sugars
281 (without MD) were similar to those obtained for pure sucrose (Roos, 2002) and trehalose
282 (Cardona *et al.*, 1997). As expected, the addition of maltodextrin (MD) caused an
283 increase in the T_g values, both for sucrose and trehalose systems (**Figure 1b**). As
284 shown in **Figure 1b** the maintenance of the glassy state (at room temperature) is limited
285 to low relative humidities: 33 % RH for trehalose model systems, 43 %RH for trehalose-

286 MD, 22 % RH for sucrose-MD, and 11 % RH for sucrose. Sucrose samples completely
287 crystallized at 43 % RH (not shown).

288 In order to investigate the influence of the different formulation components on
289 the molecular mobility of the systems, transverse relaxation times (T_2) were determined
290 by ^1H NMR after the application of a single 90° pulse (FID method). According to several
291 authors, this fast decaying T_2 was attributed to protons from solid polysaccharides and
292 from water molecules that are strongly interacting with the solid matrix by hydrogen
293 bonding (Kalichevsky & Blanshard, 1992; Ruan *et al.*, 1999; Rugraff *et al.*, 1996). To
294 analyze the effect of the physical state of the material on the molecular mobility, T_2
295 relaxation times were evaluated at different temperatures (T) between 15 and 45 $^\circ\text{C}$, at
296 all the RHs studied, and they were plotted as a function of the variable $T-T_g$ (**Figure 2**). It
297 can be observed that in the glassy state ($T-T_g < 0$) there is a slight increase in the T_2
298 values for all the studied model systems. In the case of sugar formulations, above (T -
299 T_g)= 10 $^\circ\text{C}$ the T_2 values increase considerably, particularly the sucrose system at 33 %
300 RH (**Figure 2b**). Ablett *et al.*, (1993) analyzed the evolution of T_2 (measured by FID
301 method) with temperature in relation to T_g for various malto-oligomers. They observed
302 that T_2 relaxation times were initially in the rigid lattice region at temperatures below T_g ,
303 indicating that solute molecules had NMR properties typical of a solid. Above T_g , T_2
304 values increased with increasing temperature, showing a correlation between the
305 temperature limit for rigid lattice behavior and T_g . Sugar-MD systems showed similar
306 (slightly higher) T_2 values when compared to corresponding sugar system in the glassy
307 state; and did not show an important increase above T_g at the temperatures studied. The
308 presence of maltodextrin in the formulation reduced the increase in T_2 with RH,

309 particularly for sucrose systems at 22 and 33 % RH. This could be due to the high
310 molecular weight of the polymer and higher T_g values, compared to the pure sugars.

311 Citral sensory differences in the encapsulated spray-dried matrices showed that
312 the six pairs of samples (T – TM; T – S; T – SM; S – TM; TM – SM; S – SM) analyzed by
313 paired comparison test were not perceived as different (data not shown).

314 In order to quantify the perceived aroma by the different matrices a calibration
315 curve of citral solutions was made and the sensory perceived citral was expressed in
316 percentage (weight / volume). **Figure 3** shows that citral retention after spray-drying was
317 similar for all the model systems, being slightly higher for the SM model system.

318 Citral retention was evaluated in the different powders by gas chromatography
319 and it was compared with sensory evaluation data. **Figure 3** shows that citral retention
320 after spray-drying was similar for all the model systems, being slightly higher for the TM
321 model system.

322 This result suggests that citral retention for sucrose and trehalose matrices was
323 about the same in quantity and quality, at the conditions used in the present work.

324 A slight divergence was observed between sensory and chromatographic results,
325 i.e. tendency to slightly higher aroma retention for SM model system in sensory data,
326 and for TM model system in gas chromatography determinations. These differences
327 were not significant and both may be considered the same within methodological error.

328 Analytical studies in fruit purees reported by Komes *et al.* (2003) suggested that
329 trehalose can entrap volatiles in a more effective way than sucrose; however very
330 recently, Galmarini *et al.* (2011) showed that sucrose, MD and trehalose have different
331 abilities to entrap different volatiles. Galmarini *et al.* (2011) found that the aroma profile
332 of reconstituted freeze dried strawberry puree previously added with trehalose, sucrose

333 or MD, (or combinations) indicated that those sugars had different abilities for retention
334 of volatiles having high or low molecular weight.

335

336 **4. Conclusions**

337 Sensory and chromatography results showed that citral retention after spray
338 drying was similar for both trehalose and sucrose matrices; however, physical stability of
339 trehalose formulations was better as compared to sucrose. This suggested that
340 trehalose could be successfully used as a replacement for sucrose in spray-dried citral
341 formulations.

342

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348

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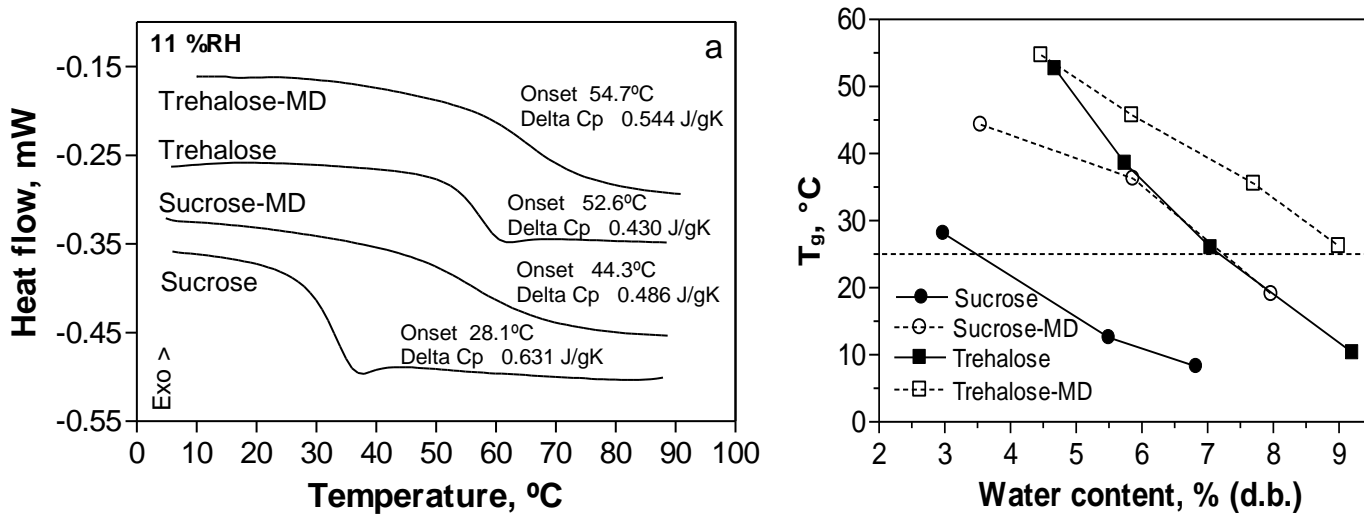


Figure 1: DSC thermograms showing the glass transition of the different powders equilibrated at 11 % RH (a), and glass transition temperature (onset) as a function of water content (b). Dashed line indicates room temperature (25°C).

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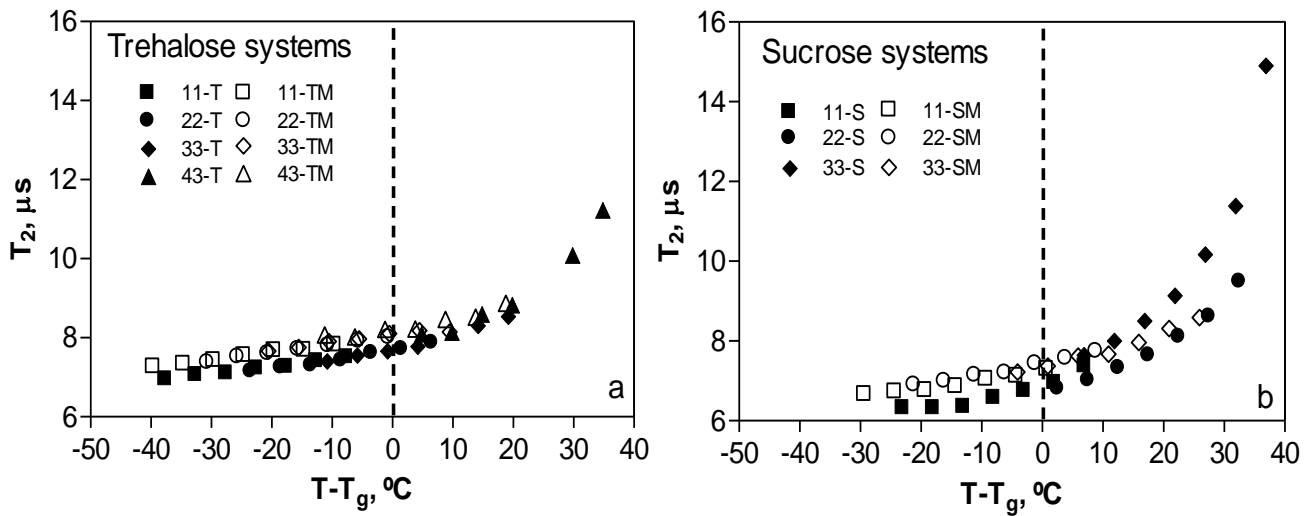
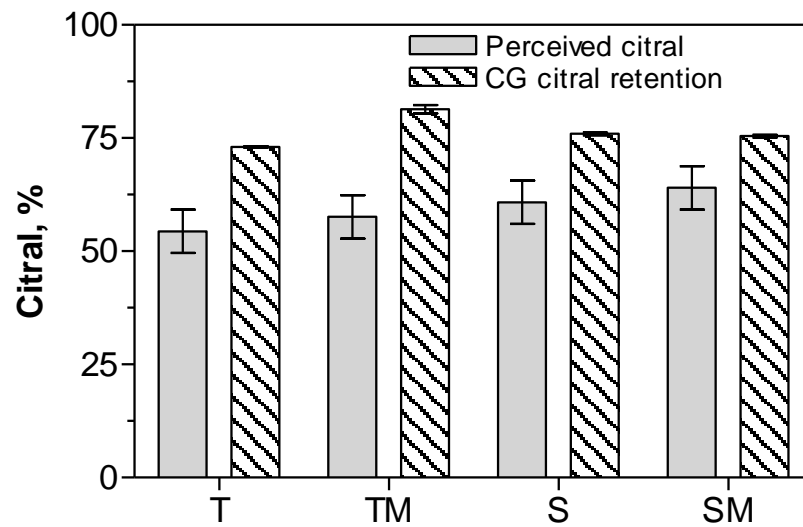


Figure 2. T₂ relaxation times obtained by ¹H NMR as a function of the variable (T-T_g), where T is the measurement temperature for NMR, and T_g is the glass transition temperature at the different relative humidities analyzed: a) trehalose systems; b) sucrose systems.

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Figure 3. Percentage of citral retention (GC) and perceived citral (sensory evaluation) in the different formulations: Trehalose (T), Trehalose-MD (TM), Sucrose (S), Sucrose-MD (SM). Non significant differences were observed both for GC and sensory evaluation studies ($\alpha < 0.05$).

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525 **Table 1.** Pseudo-equilibrium water content (% in dry basis) of the formulations at
526 different relative humidities (RH).

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Formulation	RH (%)			
	11	22	33	43
T	4.68 ± 0.01	5.74 ± 0.01	7.05 ± 0.02	9.21 ± 0.05
S	2.98 ± 0.01	5.51 ± 0.03	6.83 ± 0.12	crystallized
TM	4.47 ± 0.24	5.85 ± 0.24	7.70 ± 0.20	9.00 ± 1.23
SM	3.56 ± 0.16	5.87 ± 0.20	7.97 ± 0.09	crystallized