

Real-time monitoring of fat crystallization using pulsed acoustic spectroscopy and supervised machine learning

*Original*

Real-time monitoring of fat crystallization using pulsed acoustic spectroscopy and supervised machine learning / Metilli, L., Morris, L., Lazidis, A., Marty-Terrade, S., Holmes, M., Povey, M., Simone, E.. - In: JOURNAL OF FOOD ENGINEERING. - ISSN 0260-8774. - ELETTRONICO. - 335:(2022), p. 111192. [10.1016/j.jfoodeng.2022.111192]

*Availability:*

This version is available at: 11583/2969868 since: 2022-07-07T19:30:33Z

*Publisher:*

Elsevier Ltd

*Published*

DOI:10.1016/j.jfoodeng.2022.111192

*Terms of use:*

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

*Publisher copyright*

Elsevier postprint/Author's Accepted Manuscript

© 2022. This manuscript version is made available under the CC-BY-NC-ND 4.0 license  
<http://creativecommons.org/licenses/by-nc-nd/4.0/>. The final authenticated version is available online at:  
<http://dx.doi.org/10.1016/j.jfoodeng.2022.111192>

(Article begins on next page)

1 Real-time monitoring of fat crystallization using  
2 pulsed acoustic spectroscopy and supervised machine  
3 learning

4 *Lorenzo Metilli<sup>1</sup>, Liam Morris<sup>1</sup>, Aris Lazidis<sup>2</sup>, Stephanie Marty-Terrade<sup>3</sup>, Melvin Holmes<sup>1</sup>,*  
5 *Megan Povey<sup>1</sup> and Elena Simone<sup>1,4</sup>*

6 <sup>1</sup> School of Food Science and Nutrition, Food Colloids and Bioprocessing group, University of  
7 Leeds, Woodhouse Lane, Leeds LS2 9JT, UK

8 <sup>2</sup> Nestlé Product Technology Centre Confectionery, Haxby Road, York YO31 8TA, UK

9 <sup>3</sup> Nestlé Research, Vers-chez-les-Blanc, 1000 Lausanne 26, Switzerland

10 <sup>4</sup> Department of Applied Science and Technology (DISAT), Politecnico di Torino, Torino (Italy)

11

12 KEYWORDS

13 Crystallization, Fats, Oils, Ultrasound, Machine Learning

14

15 ABSTRACT

16

17 Enhancing the control and yield of lipid crystallization is fundamental in several industrial areas,  
18 including pharmaceutical, cosmetic and food manufacturing. However, the multi-component  
19 nature of fats and oils poses a challenge in the understanding and control of the final product  
20 properties. While the crystallization of lipid has been extensively studied with offline techniques,  
21 online monitoring of the process would be highly advantageous, especially in large-scale sheared  
22 vessels. In this work, a novel method to calculate the solid fat content (SFC%) of crystallizing  
23 lipids under shear, based on an acoustic probe and supervised-machine learning, is presented. The  
24 temperature, composition and ultrasonic velocity of the samples, and the SFC(%) measured with  
25 nuclear magnetic resonance were used to develop a predictive model to calculate the SFC(%)  
26 during crystallization. Gaussian models showed the highest accuracy compared to linear and  
27 regression tree models (RMSE = 0.03 vs 0.7 and 0.25, respectively).

## 28 **1. Introduction**

29 The crystallization of lipids is a fundamental unit operation for several manufacturing industries,  
30 including pharmaceuticals (Jose & Netto, 2019), cosmetic (Duprat-De-Paule et al., 2018; Patel et  
31 al., 2021) and food (Rios et al., 2014). Recently, the use of crystalline fat to produce structured  
32 oils (oleogels) has gained significant attention from academia and industry alike, due to the  
33 potential to produce solid-like materials with low amounts of saturated fat and specific  
34 macroscopic properties (Patel & Dewettinck, 2016). Furthermore, oleogels may be used as  
35 precursors for the production of oil-continuous foams (Binks & Vishal, 2021) and emulsified oil  
36 foams (Brun et al., 2015; Goibier et al., 2019) that can find application as nutrients or drug delivery  
37 vehicles, or as structuring materials for food products. In all of the above examples, the properties  
38 of the fat crystals, such as crystal size, shape and polymorphism, significantly affect the stability  
39 and functionality (*e.g.*, drug delivery, oil binding capacity, air incorporation) of the final oleogel

40 material (Co & Marangoni, 2012; Heymans et al., 2017). The total amount of crystals, *i.e.* the solid  
41 fat content (*SFC%*), is a general parameter strongly related to some macroscopic properties of fat-  
42 based materials, such as the melting point, hardness and texture (Himawan et al., 2006). Hence,  
43 monitoring *SFC%* during crystallization processes is important to determine when equilibrium  
44 conditions are reached, and therefore maximize the yield of crystallization in industrial contexts.

45 The *SFC%* is routinely measured by means of nuclear magnetic resonance (NMR) (Cerdeira et al.,  
46 2004), differential scanning calorimetry (DSC) (Foubert et al., 2008) or small-angle X-Ray  
47 scattering (SAXS) (Ladd Parada et al., 2019). These techniques, however, are all off-line, requiring  
48 the collection of a sample (not always a trivial operation, especially if the sample melting point is  
49 close to ambient temperature) and some degree of sample preparation, which can significantly  
50 affect the measurement. In the context of industrial large-scale crystallization, the issues related to  
51 off-line analysis and sampling are tackled by applying process analytical technology (PAT) tools,  
52 which enable real-time monitoring of the product properties, enhanced process understanding, and  
53 the application of the so called “Quality by Design” (QbD) strategy (Rathore et al., 2010).  
54 Common PAT tools used to monitor crystallization usually include *in situ* probes that exploit the  
55 scattering or absorption of electromagnetic radiation (visible light, ultra-violet (UV) or infra-red)  
56 by the sample to monitor phase transitions, polymorphic transformations or crystal morphology  
57 (Hansen et al., 2017; Simone et al., 2015; Simone et al., 2019). In the case of industrial lipid  
58 crystallization, online determination of the *SFC%* is highly sought, albeit presenting some  
59 challenges. Fat crystals form a viscous three-dimensional network of aggregates with a fractal  
60 pattern (Tang & Marangoni, 2008), whose quantification is non-trivial. Moreover, (partially)  
61 crystalline fat is often opaque to electromagnetic radiation, limiting the analysis to the surface of  
62 the sample. While oleogels (and hydrogels) are widely used in consumer products, at present there

63 are sparse examples of PAT tools applied to the manufacturing process of materials with similar  
64 properties (Bostijn et al., 2018; Pu et al., 2015).

65 The use of low-power ultrasound for studying the crystallization of lipids has been proposed as a  
66 non-invasive, non-destructive method since the 1980s (Hussin & Povey, 1984; McClements &  
67 Povey, 1988; McClements & Povey, 1987). Low-power ultrasound, *i.e.*, sound waves exceeding  
68 20 kHz frequency, can penetrate opaque media, without causing physical and chemical changes in  
69 the sample. Moreover, it is relatively inexpensive compared to other spectroscopy techniques, and  
70 it is easily adaptable to different measuring configurations (Povey, 2017). The technique involves  
71 the propagation of a short (few microseconds) ultrasonic pulse from a transducer into the sample;  
72 this pulse is received by either another transducer on the other side of the measuring apparatus  
73 (pitch-and-catch mode) or it is reflected and received by the same emitting transducer (pulse-echo  
74 mode). The velocity of sound ( $c_{\text{sample}}$ ) is then calculated from the travelled path length as a function  
75 of time and temperature. The acoustic attenuation ( $\alpha$ ), *i.e.*, the ratio of the amplitude of the sent  
76 and received pulse, may also be calculated. As both the velocity of sound and acoustic attenuation  
77 depend on the physicochemical properties of the sample, such as density and adiabatic  
78 compressibility, and the presence of heterogeneities, acoustic measurements can be used to  
79 monitor phase transitions such as crystallization and polymorphic transformations (Fairley &  
80 McClements, 1992; Kloek et al., 2000; Miles et al., 1985). Several authors demonstrated the use  
81 of custom-made acoustic cells to study fat crystallization using acoustic signals, with particular  
82 emphasis on determining the *SFC*(%) (Birkhofer et al., 2008; Martini et al., 2005a, Martini et al.,  
83 2005b; Singh et al., 2002, Singh et al., 2004). Nevertheless, most of the previous works on fat  
84 crystallization were carried out in quiescent conditions, and/or with small sample volumes, thus

85 excluding the effect of shear and secondary nucleation on crystallization which are predominant  
86 on industrial scale (Agrawal & Paterson, 2015).

87 Despite its several advantages and ease of implementation, there are only sporadic examples in the  
88 literature on the use of acoustic probes as a PAT tool for studying lipid crystallization. The  
89 immersion probe described in Titiz-Sargut & Ulrich (2003), which featured two 2 MHz  
90 transducers in pitch-and-catch mode, was applied to the determination of the metastable zone  
91 width (MSZW) of coconut oil, and validated by optical back-reflectance measurements (ORM)  
92 (Chaleepa et al., 2010). The authors focused their study on the effect of different levels of shear,  
93 cooling rates and the presence of additives on the MSZW; however, no quantitative information  
94 on the *SFC%* of this lipid system was reported. Due to the complexity involved in the  
95 crystallization of lipids, such as the occurrence of melt-mediated polymorphic transformations,  
96 and the development of crystalline networks whose size range from nanometres to several  
97 hundreds of microns, the determination of the *SFC%* directly from acoustic parameters is non-  
98 trivial. Moreover, the large acoustic attenuation exhibited by crystalline fat results often in loss of  
99 the acoustic signal (Rigolle et al., 2018). One of the growing trends in the use of PAT tools is the  
100 implementation of machine learning (ML) algorithms to facilitate analysis of real-time data  
101 provided by sensors, and to enable prediction of material properties of interest based on training  
102 the algorithm with known outcomes (supervised machine learning) (Wasalathanthri et al., 2020).  
103 Examples of ML applications in the context of crystallization include the automatic detection of  
104 crystal aggregation from microscopic images (Ochsenbein et al., 2015), the real-time estimation  
105 of the crystal size distribution of 2D needle-shaped crystals from measurements of chord length  
106 and aspect ratio distributions (Szilágyi & Nagy, 2018) and the estimation of the 3D size  
107 distribution of plate-like particles using projections from multiple cameras (Jaeggi et al., 2021).

108 The approach can be extended to the actual control of crystallization processing, for example  
109 through the use of convolutional neural network (CNN) feedback control to dissolve undesired  
110 precipitated impurities during the crystallization of active pharmaceutical ingredients (APIs)  
111 (Salami et al., 2021). Recently, ML has been applied to ultrasonic reflectance measurements to  
112 monitor the mixing process in a large-scale vessel (Bowler et al., 2020; Bowler & Watson, 2021).

113 In this work, a novel technique for estimating the *SFC%*, based on a custom-built acoustic probe  
114 (Morris et al., 2021) and supervised machine learning is presented. This immersion acoustic probe  
115 was used as a PAT sensor to monitor the crystallization of a cocoa butter/sunflower oil oleogel  
116 system, in a 1L scale vessel and under shear. Stirring was maintained constant throughout the  
117 whole temperature profile. Cocoa butter and sunflower oil are both ingredients widely used in  
118 food, cosmetic and pharmaceutical applications (Metilli et al., 2021). The ultrasonic data was  
119 validated with light turbidimetry, and the *SFC%* of the crystallized oleogel was measured with  
120 offline pulsed NMR (*p*NMR) at equilibrium conditions and specific temperatures. Finally,  
121 supervised machine learning was applied to develop a predictive model based on the acoustic  
122 parameters and the results of *p*NMR, enabling the calculation of *SFC%* based on the velocity of  
123 sound, sample composition and temperature.

## 124 **2. Materials and Methods**

### 125 **2.1 Cocoa butter–based oleogels**

126 Refined, bleached and deodorized cocoa butter (CB) and high-oleic sunflower oil (HOSO) were  
127 kindly provided by Nestlé PTC Confectionery (York, UK) and used without any further  
128 purification. CB was melted at 65 °C for one hour, and then mixed with HOSO at 9%, 11%, 13%  
129 and 15% concentration by weight. HOSO contains usually the following fatty acids (by weight):

130 86% oleic acid, 5% stearic acid, 3% linoleic acid, 3% palmitic acid, 1.5% behenic acid, and 0.7%  
131 arachidic acid. CB normally contains by a weight about 26% palmitic acid, 36% stearic acid, 34%  
132 oleic acid, 2.7% linoleic, and 0.9% arachidic acid.

## 133 **2.2 Fat crystallization rig**

134 The CB-HOSO mixture (900 g) was transferred to a jacketed crystallization vessel (capacity *ca.* 1  
135 L, diameter 15 cm) (Radley, UK) connected to a Huber Ministat 230 thermostat (Huber, Germany),  
136 filled with silicone oil as a heating/cooling medium. The sample was stirred continuously at 200  
137 rpm with a DLH overhead stirrer (VELP Scientifica, Italy), equipped with an anchor-shaped mixer  
138 (8 cm diameter). A Pt-100 temperature probe (Omega Engineering, UK), placed in the vessel, was  
139 used to monitor the sample temperature during the experiment. The crystallization process was  
140 followed using a Control 4000 turbidity meter (Optek, Germany) fitted with an ASD12-N  
141 absorption probe, which measured light transmittance and absorbance. Finally, the velocity of  
142 sound and the acoustic attenuation of the crystallizing mixture were measured using a custom  
143 acoustic probe, recently described in literature (Morris et al., 2021), with some design  
144 modifications. Briefly, the probe comprised a 2.25 MHz broadband transducer coupled with a  
145 Rexolite buffer rod (Sonatest model RDT5025, Sonatest, UK), and a stainless-steel acoustic  
146 reflector plate. This probe was manufactured in an ‘L’ shape configuration. The probe was  
147 connected to a UT320 pulser/receiver (UTEX scientific instruments inc., Canada) and a HDO3034  
148 digital oscilloscope (Teledyne LeCroy, USA). A schematic of the equipment is shown in Figure  
149 1.

150 The thermal profile of the experiment was set to the following: Equilibration of the fat blend  
151 mixture at 45 °C for 10 minutes, cooling to 0 °C at a nominal rate of –0.5 °C/min and holding at

152 0°C for 3 hours. The sample was then heated back to 45 °C at 1 °C/min. The process temperature  
153 and the acoustic waveforms were collected using an in-house script developed with  
154 MATLAB2021a (MathWorks, USA). Measurements were collected every 10 seconds. Each  
155 experiment was repeated three times.

## 156 **2.3 Determination of the acoustic parameters**

### 157 **2.3.1 Velocity of Sound**

158 Figure contains a diagram describing the design of the acoustic probe, and an example of  
159 waveform acquired from the oscilloscope with MATLAB.

160 The ultrasonic pulse generated from the transducer travels through the buffer rod, and it is partially  
161 reflected at the buffer rod/sample interface, due to the acoustic impedance mismatch (*i.e.*, the  
162 difference in the product of density and velocity of sound of the two materials). The pulse is then  
163 received back by the transducer after a time  $\Delta t_1$ , shown in Figure as the blue trace. Part of the  
164 initial pulse, however, is transmitted through the sample, and it is reflected by the stainless-steel  
165 reflector to the transducer after a time  $\Delta t_2$  (Figure , red trace). In order to calculate the velocity of  
166 sound in the sample, the time of flight in the sample is required. To calculate it, the initial value of  
167  $\Delta t_1$  and  $\Delta t_2$  were first determined with MATLAB from the original waveform, using the leading-  
168 edge method (*i.e.*, detecting the arrival time of the pulse envelope when it crosses a set voltage  
169 threshold). Afterwards, the shifts of the pulses' position ( $\Delta\Delta t_1$  and  $\Delta\Delta t_2$ ) during the experiment  
170 were calculated with the cross-correlation function (*xcorr*) implemented in MATLAB. This  
171 function provides an estimate of the correlation between each analysed waveform and a reference  
172 waveform, returning the intensity of the correlation value as a function of time units. The position  
173 of the maximum peak of this calculated vector corresponds to the time delay between two pulses.  
174 This method proved to be more robust in the analysis of the set of waveforms compared to applying

175 the leading-edge technique on all collected waveforms. This is because the pulse envelop was  
 176 subject to distortion due to frequency dependent signal attenuation through the experiment. The  
 177 variation in the sample time of flight ( $\Delta\Delta t_3$ ) was then calculated with Equation 1:

$$\Delta\Delta t_3 = \Delta\Delta t_2 - \Delta\Delta t_1 \quad (\text{Eq. 1})$$

178 In order to obtain an accurate value of the velocity of sound, however, it was necessary to  
 179 determine the path length dependence on the temperature with a calibration experiment. The  
 180 velocity of sound in distilled water ( $c_{\text{water}}$ ) with respect to temperature ( $T$ ) may be calculated using  
 181 a fifth-order polynomial, as first described by Chávez et al. (1985) (Equation 2):

$$c_{\text{water}} = 3.16 \cdot 10^{-9}T^5 - 1.48 \cdot 10^{-6}T^4 + 3.35 \cdot 10^{-4}T^3 - 5.81 \cdot 10^{-2}T^2 + 5.04 \cdot T + 1.40 \cdot 10^3 \quad (\text{Eq. 2})$$

182 The shift in the time of flight in distilled water ( $\Delta\Delta t_{3,\text{water}}$ ) was then measured between 50 °C and  
 183 5°C, using a  $-0.01$  °C/min cooling rate to allow the probe to reach thermal equilibrium with the  
 184 surrounding medium. The corresponding experimental path length ( $L_{\text{calibrated}}$ ) was calculated with  
 185 Equation 3

$$L_{\text{calibrated}} = \Delta\Delta t_{3,\text{water}} \times c_{\text{water}} \quad (\text{Eq. 3})$$

186 The dependence of the path length on the temperature was then obtained by fitting a fifth-order  
 187 polynomial to  $L_{\text{calibrated}}$  against the temperature (Figure ).

188 The coefficients estimated for the path length calculation were then used to accurately calculate  
 189 the velocity of sound during the crystallization experiments with Equation 4

$$c_{\text{sample}} = \frac{L_{\text{calibrated}}}{\Delta\Delta t_3} \quad (\text{Eq. 4})$$

### 190 **2.3.2 Acoustic Attenuation**

191 The acoustic attenuation quantifies the acoustic power absorbed and scattered by the sample, and  
 192 is affected by several factors, including the onset of phase transitions and scattering phenomena

193 generated by the presence of dispersed objects in the sample (McClements & Povey, 1992). In this  
194 work, the acoustic attenuation was used to detect the onset of crystal growth and dissolution during  
195 the crystallization experiments. The acoustic attenuation was calculated according to Equation 5

$$\alpha = -20 \log \frac{A}{A_0} \quad (\text{Eq. 5})$$

196 where  $A$  and  $A_0$  are the peak-to-peak amplitudes of the sample signal during the experiment, and  
197 at the start of the experiment, respectively.

198

### 199 **2.3.3 Solid Fat Content**

200 The solid fat content ( $SFC\%$ ), defined as the mass fraction of solid to liquid material in a fat blend,  
201 was calculated by applying a prediction model, developed with the Regression Learner App in  
202 MATLAB2021a, (MathWorks, USA). Regression Learner is a supervised machine-learning  
203 utility, where a regression algorithm is applied to an observation matrix and compared with a  
204 response matrix. The observation matrix comprised the temperature ( $T$ ), velocity of sound in the  
205 sample ( $c_{sample}$ ) and the amount of added cocoa butter ( $CB\%$ ) for each crystallization experiment;  
206 the response matrix contained the  $SFC\%$  values, measured with pNMR, between 5 and 45 °C for  
207 the respective samples. The velocity of sound and temperature of pure sunflower oil were also  
208 added to the observation matrix, with a corresponding solid fat content of 0%. The sunflower oil  
209 data was included in the model to provide information on the behaviour of a sample without any  
210 crystallizing material in the explored experimental conditions. Three models available in the  
211 Regression Learner App were tested for training of the dataset, and their predictive ability were  
212 compared: “Linear”, “Fine Tree” and “Gaussian Process Regression–Rational Quadratic”. The  
213 Linear model uses a linear regression to fit the data from the observation matrix. The “Fine Tree”  
214 model, instead, is a type of nonlinear model based on regression trees, which applies a recursive

215 partition of the observation matrix to improve the prediction of the response value. Lastly, the  
216 Gaussian Process Regression model, which is also nonlinear, works by predicting the probability  
217 distribution of responses for each parameter in the observation matrix. Model cross-validation was  
218 performed using the in-built function in the Regression Learner App (5-fold validation setting).  
219 Briefly, the software divides the dataset into a number of sub-sets of the same size, trains the  
220 predictive model on all sub-sets except one, which is used as test data. This process is repeated  
221 until all sub-sets have been used as test data once (Bosnić & Kononenko, 2009). The accuracy of  
222 all iterations is calculated as  $R^2$ , RMSE and other statistical parameters. In the discussion, the Root  
223 Mean Square Error values (RMSE) were compared to select the most accurate predictive model.  
224 In addition to the predictive model, an equation that estimates the equilibrium *SFC%* as function  
225 of the temperature and the concentration of CB in sunflower oil was determined using the *Curve*  
226 *Fitting Tool* in MATLAB2021a. A custom equation based on literature was used to fit the  
227 experimental data using the *NonlinearLeastSquares* method;  $R^2$ , RMSE and 95% confidence  
228 intervals for each estimated parameters were also calculated.

229

#### 230 **2.4 Pulsed Nuclear Magnetic Resonance (pNMR)**

231 The solid fat content (*SFC%*) of the CB-HOSO mixtures was determined with *pNMR* using a  
232 Bruker Minispec NMR (Bruker, Switzerland). The samples were collected at the end of the  
233 crystallization experiment, transferred to a 10 mm inner diameter NMR tube and stored in a fridge  
234 at 4 °C. The *SFC%* was measured between 5 and 45 °C, in steps of 5 °C. During the experiment,  
235 the NMR tube was left to equilibrate for 90 minutes for each temperature step. The measurements  
236 were carried out in triplicates. The resulting *SFC(%)* vs. temperature data was fitted using a  
237 Gompertz-type model (Farmani, 2015) to obtain the *SFC(%)* as a function of the temperature and  
238 CB%.

### 239 3. Results and Discussion

#### 240 3.1 Fat crystallization monitored by PAT tools

241 The crystallization of one 9% CB w/w in HOSO sample, monitored with turbidity and the acoustic  
242 probe, is shown in Figure 4.

243 While some variability among the three experiments conducted for each CB concentration was  
244 observed, (particularly in the crystallization temperature and the absolute values of acoustic  
245 attenuation) the trends observed for all CB/HOSO samples were similar. In particular, for the  
246 experiment shown in Figure 4a, four regions could be identified. Between 0 and 115 minutes  
247 (region I of Figure 4a) the temperature of the sample followed the cooling profile, from 45 °C to  
248 5°C, accompanied by an increase in the velocity of sound, from 1390 to 1510 m/s, due to the  
249 negative velocity coefficient with respect to temperature (McClements & Povey, 1992). After 115  
250 minutes, when the sample temperature reached 5 °C, the onset of nucleation was detected by a  
251 sharp decrease in the light transmittance, due to the sample becoming turbid (region II).  
252 Simultaneously, the light absorbance increased, exhibiting two distinct steps: a first, modest  
253 increase occurring between 115 and 129 minutes, and a larger increase after 129 minutes.  
254 Interestingly, the acoustic attenuation and the velocity of sound were responsive to the second step  
255 only, with a delay in detecting the onset of crystallization of *ca.* 14 minutes. This behaviour was  
256 consistently observed across all CB % w/w concentrations (Figure S1 of Supporting Information),  
257 and reported also in previous works (Martini et al., 2005b; Singh et al., 2002). The sheared  
258 crystallization of CB/HOSO mixtures was thoroughly investigated in a recent publication,  
259 reporting that cocoa butter crystallized as spherical aggregates of crystalline nanoplatelet (CNPs)  
260 in the  $\beta(V)$  form (Metilli et al., 2021) (Figure 5). Additional characterization is provided in the  
261 Supporting Information (Figure S2).

262 It is hypothesized, however, that CB first nucleated as a metastable polymorph ( $\alpha$  or  $\beta'$ ) and then  
263 transformed into the  $\beta(V)$  structure during the experiments. It might be that the change in  
264 compressibility and/or density associated to the formation of a liquid crystal structure – most likely  
265 the  $\alpha$  polymorph – were too small to be detected by the acoustic probe used in this work (Ladd  
266 Parada et al., 2019). On the other hand, the nucleation and the growth of the  $\beta(V)$  crystals and the  
267 consequent development of the fat crystal network were clearly detected in both the velocity of  
268 sound and acoustic attenuation (at 129 minutes). As the velocity of sound depends strongly on the  
269 temperature, the first derivative with respect to temperature was calculated to better discriminate  
270 the effect of crystal nucleation on this acoustic parameter (Figure 4b). Before the appearance of  
271 fat crystals, when the change in the velocity of sound was only dependent on the temperature, the  
272 calculated first derivative was zero; whereas, upon growth of the CB crystal network, the value of  
273 this parameter changed significantly. Variations in the first derivative occurred at the same time  
274 with the increase of the acoustic attenuation, which was caused by (a) scattering by fat crystals and  
275 (b) additional attenuation mechanisms associated with the space filling interconnected fat crystals,  
276 which also affected the overall compressibility of the system through the appearance of an  
277 additional ‘frame modulus’ (Povey, 2017). Finally, fat crystal nucleation might release a high  
278 amount of latent heat of solidification. In the case of the experiment of Figure 4a, such exothermic  
279 process increased the process temperature from 3.2 °C to 5.65 °C (130-137 minutes). While this  
280 temperature perturbation did not affect the signal from the turbidity probe or the acoustic  
281 attenuation, it did affect the velocity of sound, which is strongly dependent on the sample  
282 temperature.

283 After nucleation of the  $\beta(V)$  polymorph, the crystallization process of Figure 4a proceeded until  
284 275 minutes (region III), when the sample temperature reached *ca.* 1.5 °C, which corresponded to

285 the thermal equilibrium of the whole setup. In this time the acoustic attenuation kept increasing,  
286 following a similar trend to the light absorbance. The velocity of sound also increased, but mostly  
287 due to the change in temperature. Nevertheless, it is worth noticing in Figure 4b that the appearance  
288 of fat crystals determined an increase in the noise of the first derivative of the velocity of sound,  
289 albeit its mean value was still around zero.

290 After reaching equilibrium at around 275 minutes, the sample was heated back to 45 °C (region  
291 IV). A focus on this region of the experiment is displayed in Figure 4c. In the heating step between  
292 1.5 °C to 15 °C (from 280 minutes to 315 minutes), a decrease in the velocity of sound linearly  
293 proportional to the decrease in temperature was observed. At the same time, a moderate decrease  
294 in the acoustic attenuation happened, potentially linked to a decrease in the *SFC%* of the  
295 crystallized sample.

296 Further increase in the sample temperature (from 315 to 330 minutes, 15 °C to 25 °C) resulted in  
297 the complete melting of the fat crystalline network, which was evident in the sharp increase in  
298 light transmittance and in the decrease in light absorbance, as well as acoustic attenuation. It is  
299 worth noticing that both techniques detected full dissolution at the same time, around 327 minutes.  
300 The velocity of sound presented a steeper decline in correspondence to the full melting; this was  
301 clearly observable in the calculated first derivative.

302 Upon complete remelting of the sample (330 minutes – 350 minutes) all parameters returned to  
303 their values prior to crystallization.

304 This set of experiments showed that the tested acoustic probe could be used to monitor  
305 crystallization processes, although it seemed less sensitive than light turbidimetry to the detection  
306 of early nucleated crystals, perhaps due to their liquid crystalline nature.

307 Experiments carried out with a higher weight % of CB showed similar trends for the acoustic and  
308 light signals. The velocity of sound of the crystallized oleogels at different % w/w of added CB,  
309 between 5 and 35°C, is shown in Figure 6.

310 In agreement with previous literature (McClements & Povey, 1992), the velocity of sound of the  
311 crystallized oleogels increased, for the same range of temperatures, with increasing CB % w/w  
312 (approximately by 10 m/s every 2 % of CB). From Figure 6 it can be noted that, between 20 and  
313 25°C, the velocity of sound of all samples decreased steeply, due to the oleogels melting. It can be  
314 appreciated that such decrease occurred at lower temperatures for lower CB concentrations, in  
315 accordance with the lower melting point of the oleogels. Once liquid, the oleogels then displayed  
316 the same velocity of sound of the sunflower oil.

317 The acoustic attenuation measured at equilibrium also increased with the amount of CB contained  
318 in the oleogel, as shown in **Table 1**. In fact, higher concentrations of crystalline solids scattered  
319 more sound and attenuated it. The relatively large standard deviation of the acoustic attenuation  
320 for the 9% CB w/w sample, and its non-linear increase with higher concentrations of CB w/w,  
321 however, prompts further work to establish a more robust relationship with the amount of solid fat  
322 in the sample. In fact, the relationship between acoustic attenuation, *SFC*(%) and the crystal size  
323 and shape is challenging to elucidate (Martini et al., 2005a).

324 The solid fat content (*SFC*%) of the CB/HOSO samples as a function of temperature was measured  
325 with *p*NMR at the end of the crystallization step (Figure 7). Samples were collected directly from  
326 the vessel to ensure direct comparison with the performed experiments.

327 By inspecting the *SFC*% values at 5 °C, it can be noted that all samples displayed a lower *SFC*%  
328 compared to the total amount of added cocoa butter. This is because the main triacylglycerides  
329 species that are solid at ambient temperature, 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), 1,3-

330 distearoyl-2-oleoyl-glycerol (SOS) and 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS),  
331 constitute about 80% w/w of cocoa butter, while the remaining 20% comprises mono- and di-  
332 glycerides, phospholipids, sterols and free fatty acids that are liquid at ambient conditions (Dimick,  
333 1991). It is worth noticing that the *p*NMR measurements present an average standard deviation of  
334 0.101%, which is a more than acceptable value for industrial applications (particularly the  
335 confectionary sector).

336 The *SFC*(%) values at equilibrium were fitted using a Gompertz-type model, similar to the one  
337 described in Farmani (2015) (Equation 6):

$$SFC(\%)(T, CB) = (b_0 + b_1 CB) e^{-e^{\frac{-(T - (b_{01} + (b_{11} CB))}{c})}} \quad (\text{Eq. 6})$$

338 where T is the sample temperature in °C, CB is the concentration (w/w) of added cocoa butter, and  
339  $b_0 = 1.24$ ,  $b_1 = 73$ ,  $b_{01} = 13.48$ ,  $b_{11} = 38.5$  and  $c = -4.66$  are the coefficient determined via  
340 interpolation in MATLAB. The  $R^2$  of the fitting was equal to 0.9965 with a *RMSE* of 0.2324%  
341 (95% confidence intervals for each parameter are shown in supporting information Table S1),  
342 which is still acceptable for industrial purposes.

343 This fit enabled calculation of the equilibrium *SFC*(%) as a function of temperature and CB  
344 concentration, which can be used in combination with the predictive models determined in the next  
345 section for oleogels with composition ranging from 9 to 15% of CB.

346

### 347 **3.2 Solid fat content predicted by Regression Learner model**

348 The measured *SFC*(%) with *p*NMR, and the predicted *SFC*(%) obtained with the Regression  
349 Learner models is displayed in Figure 8.

350 It can be noticed how the Linear model showed inaccuracies both when the sample was in the  
351 melted state (high temperatures), as well as in its crystallized phase, at all concentrations. This

352 could be ascribed to the non-linear dependence of the  $SFC(\%)$  with respect to temperature, as  
353 already highlighted in Figure 7. When considering the Fine Tree model, the accuracy generally  
354 improved, however still displaying some errors in predicting the  $SFC(\%)$  of the oleogels, in  
355 particular between 15 and 25 °C. Finally, the GPR model showed the best predictive accuracy, for  
356 all samples and temperatures, with an RMSE value almost one order of magnitude lower compared  
357 to the other two models. Such improvement could stem from the similarity between the Gompertz-  
358 type  $SFC(\%)$  dependence with temperature (Equation 6) and the gaussian-type distribution  
359 function (also exponential). Therefore, the GPR model was selected to predict the SFC% evolution  
360 during fat crystallization, as shown in Figure .

361 The increase in the predicted value of the  $SFC\%$  in the oleogel matched the increase in the acoustic  
362 attenuation at 130 minutes, reaching 5% over few minutes and then levelling to a value of about  
363 7.6%, very close to the equilibrium  $SFC\%$  at such temperature (as shown in Figure 7). During the  
364 heating phase, the  $SFC\%$  started decreasing, falling sharply to 0% when the temperature was raised  
365 above 20 °C and remaining constant thereafter, until the end of the experiment. Therefore, in  
366 contrast with previous research, the approach presented in this work only requires measuring  
367 velocity of sound, the temperature, and the  $SFC(\%)$  measured with  $pNMR$  on samples on the  
368 concentration of interest. The  $SFC(\%)$  is then rapidly and accurately predicted from the ultrasonic  
369 probe data under realistic operating conditions (*i.e.*, under shear and with large sample volumes).  
370 The predicted  $SFC\%$  for the samples at different CB % was plotted as a function of temperature  
371 in Figure .

372 By inspecting the cooling profile, it can be noted that crystal growth, signalled by the increase in  
373  $SFC\%$ , occurred at higher crystallization temperatures for oleogels containing higher % of CB, as  
374 also reported previously in Metilli et al. (2021) for this type of system. Whereas, by inspecting the

375 heating profile, the *SFC%* decreased with the temperature with the same trend observed with  
376 *p*NMR. The estimated values of *SFC%* at 5 °C during heating were 7.3, 8.7, 10.2 and 11.7% for  
377 the 9, 11, 13 and 15% CB samples, respectively. These values are very close to the equilibrium  
378 *SFC%* measured with *p*NMR at the same temperature (7.6, 8.5, 10.3 and 11.7%) indicating the  
379 reliability of the predictive model built. Furthermore, both *p*NMR and acoustic spectroscopy  
380 showed agreement that at 25 °C all samples returned to the full liquid state.

381 This work presents a prototype of an acoustic in situ probe, coupled with a reliable machine  
382 learning-based predictive model, for online monitoring of oleogels crystallization. The probe could  
383 provide useful quantitative information on the evolution of crystallization processes in an  
384 industrial setting with minimal investment cost and calibration experiments. Furthermore, due to  
385 the versatility of acoustic probes, the design of the hardware could be optimized in order to allow  
386 effective measurements even in different fluid-dynamic environment (e.g., different crystallizers  
387 designs, commercial tempering equipment).

388

#### 389 **4. Conclusions**

390 In this work, the crystallization of cocoa butter–based oleogels was characterized qualitatively and  
391 qualitatively using a custom-built immersion probe based on pulsed acoustic spectroscopy. The  
392 *SFC%* of the oleogel during crystallization was estimated through a predictive model developed  
393 with supervised machine learning. Such method uses the acoustic parameters (*i.e.*, velocity of  
394 sound) collected from the immersion probe, the sample temperature and composition, and the  
395 *SFC%* measured with *p*NMR. The predicted *SFC%* and its evolution during the shear  
396 crystallization of the CB/HOSO mixtures was in agreement with the nucleation and development  
397 of crystalline fat, as corroborated by light turbidimetry and acoustic attenuation. A comparison

398 between several predictive models showed that Gaussian Process Regression model was the most  
399 accurate in describing the *SFC%* both prior and during crystallization. The *SFC%* value increased  
400 steeply during crystal growth, with a final value close to 80% by weight of the added cocoa butter  
401 in the mixture, with a similar trend to the acoustic attenuation. The *SFC%* then returned to 0%  
402 close to the melting point of the oleogel, in agreement with the melting profile measured with  
403 *p*NMR. Moreover, this work highlighted that turbidimetry was more sensitive in detecting the  
404 nucleation of lipid crystals, whereas the acoustic probe was more responsive to the crystal growth  
405 process. This difference could be ascribed to the liquid-crystalline nature of the metastable lipid  
406 polymorph that developed during nucleation, for which the change in density and compressibility  
407 may be too small to be measured by the current acoustic probe. Using a different frequency  
408 transducer might increase the sensitivity of the ultrasonic probe to crystal nucleation; however,  
409 this might result in excessive signal attenuation at equilibrium conditions, at which the *SFC%*  
410 needs to be calculated. In summary, the results presented in this work demonstrate the feasibility  
411 of implementing acoustic probes as PAT tools, in combination with supervised machine learning,  
412 to improve the oleogel crystallization yield through the timely and accurate monitoring of the  
413 *SFC%*.

414 ASSOCIATED CONTENT

415

#### 416 **Supporting Information.**

417 PAT tools plot of 9, 11, 13 and 15% w/w CB in HOSO samples focused on nucleation of CB  
418 crystals (Figure S1). SAXS and WAXS patterns, DSC and CryoSEM images of a 15% w/w CB in  
419 HOSO oleogel sample (Figure S2).

420 AUTHOR INFORMATION

421

422 **Corresponding Author**

423 Prof. Elena Simone

424 Elena.simone@polito.it

425

426 **Author Contributions**

427 The manuscript was written through contributions of all authors. All authors have given approval  
428 to the final version of the manuscript.

429

430 **Funding Sources**

431 Centre for Doctoral Training in Soft Matter and Functional Interfaces (Grant ref. no.  
432 EP/L015536/1).

433 Royal Society (Grant ref. no. INF\R2\192018).

434 Royal Academy of Engineering (Grant ref. no. IF/192031).

435 **ACKNOWLEDGMENTS**

436 The authors would like to acknowledge the Engineering and Physical Sciences Research Council  
437 funded Centre for Doctoral Training in Soft Matter and Functional Interfaces, grant ref. no.  
438 EP/L015536/1 as well as Nestlé PTC Confectionery (York, UK) for the pulsed NMR analysis, and  
439 the financial and writing support. E.S. also acknowledges Royal Society (Grant ref. no.

440 INF\R2\192018) and Royal Academy of Engineering (Grant ref. no. IF/192031) for additional  
441 funding.

#### 442 ABBREVIATIONS

443 CB cocoa butter, GPR gaussian process regression, HOSO high oleic sunflower oil, ML machine  
444 learning, SFC solid fat content, PAT process analytical technologies, *p*NMR pulsed nuclear  
445 magnetic resonance.

#### 446 REFERENCES

447 Agrawal, S. G., & Paterson, A. H. J. (2015). Secondary Nucleation: Mechanisms and Models.  
448 *Chemical Engineering Communications*, 202(5), 698–706.  
449 <https://doi.org/10.1080/00986445.2014.969369>

450 Rigolle, A., Van Den Abeele, K. and Foubert, I. (2018). Conventional and New Techniques to  
451 Monitor Lipid Crystallization. In *Crystallization of Lipids*, K. Sato  
452 (Ed.). <https://doi.org/10.1002/9781118593882.ch17>

453 Binks, B. P., & Vishal, B. (2021). Particle-stabilized oil foams. *Advances in Colloid and Interface*  
454 *Science*, 291, 102404. <https://doi.org/10.1016/j.cis.2021.102404>

455 Birkhofer, B. H., Jeelani, S. A. K., Windhab, E. J., Ouriev, B., Lisner, K.-J., Braun, P., & Zeng,  
456 Y. (2008). Monitoring of fat crystallization process using UVP–PD technique. *Flow*  
457 *Measurement and Instrumentation*, 19(3–4), 163–169.  
458 <https://doi.org/10.1016/j.flowmeasinst.2007.08.008>

459 Bosnić, Z., & Kononenko, I. (2009). An overview of advances in reliability estimation of  
460 individual predictions in machine learning. *Intelligent Data Analysis*, 13(2), 385–401.

461 <https://doi.org/10.3233/IDA-2009-0371>

462 Bostijn, N., Hellings, M., Van Der Veen, M., Vervaet, C., & De Beer, T. (2018). In-line UV  
463 spectroscopy for the quantification of low-dose active ingredients during the manufacturing  
464 of pharmaceutical semi-solid and liquid formulations. *Analytica Chimica Acta*, *1013*, 54–62.  
465 <https://doi.org/10.1016/j.aca.2018.02.007>

466 Bowler, A. L., Bakalis, S., & Watson, N. J. (2020). Monitoring Mixing Processes Using Ultrasonic  
467 Sensors and Machine Learning. *Sensors*, *20*(7), 1813. <https://doi.org/10.3390/s20071813>

468 Bowler, A. L., & Watson, N. J. (2021). Transfer learning for process monitoring using reflection-  
469 mode ultrasonic sensing. *Ultrasonics*, *115*(May), 106468.  
470 <https://doi.org/10.1016/j.ultras.2021.106468>

471 Brun, M., Delamplé, M., Harte, E., Lecomte, S., & Leal-Calderon, F. (2015). Stabilization of air  
472 bubbles in oil by surfactant crystals: A route to produce air-in-oil foams and air-in-oil-in-  
473 water emulsions. *Food Research International*, *67*, 366–375.  
474 <https://doi.org/10.1016/j.foodres.2014.11.044>

475 Cerdeira, M., Candal, R.J., Herrera, M. L. (2004). Analytical Techniques for Nucleation Studies  
476 in Lipids : Advantages and Disadvantages. *Journal of Food Science*, *69*(9), R185–R191.

477 Chaleepa, K., Szepes, A., & Ulrich, J. (2010). Metastable zone determination of lipid systems:  
478 Ultrasound velocity versus optical back-reflectance measurements. *European Journal of*  
479 *Lipid Science and Technology*, *112*(5), 565–573. <https://doi.org/10.1002/ejlt.200900225>

480 Chávez, M., Sosa, V., & Tsumura, R. (1985). Speed of sound in saturated pure water. *Journal of*  
481 *the Acoustical Society of America*, *77*(2), 420–423. <https://doi.org/10.1121/1.391861>

- 482 Co, E. D., & Marangoni, A. G. (2012). Organogels: An alternative edible oil-structuring method.  
483 *JAOCS, Journal of the American Oil Chemists' Society*, 89(5), 749–780.  
484 <https://doi.org/10.1007/s11746-012-2049-3>
- 485 Dimick, P. S. (1991). Principles of cocoa butter crystallization. *Manufacturing Confectioner*,  
486 71(5), 115–125.
- 487 Duprat-De-Paule, S., Guilbot, J., Roso, A., Cambos, S., & Pierre, A. (2018). Augmented bio-based  
488 lipids for cosmetics. *OCL - Oilseeds and Fats, Crops and Lipids*, 25(5).  
489 <https://doi.org/10.1051/ocl/2018036>
- 490 Fairley, P., & McClements, D. J. (1992). Frequency scanning ultrasonic pulse echo reflectometer.  
491 *Ultrasonics*, 30(6), 403–405.
- 492 Farmani, J. (2015). Modeling of solid fat content of chemically interesterified fully hydrogenated  
493 soybean oil and canola oil blends as a function of temperature and saturated fatty acids.  
494 *Journal of Food Measurement and Characterization*, 9(3), 281–289.  
495 <https://doi.org/10.1007/s11694-015-9233-8>
- 496 Fernández-Moya, V., Martínez-Force, E., & Garcés, R. (2000). Identification of triacylglycerol  
497 species from high-saturated sunflower (*Helianthus annuus*) mutants. *Journal of Agricultural*  
498 *and Food Chemistry*, 48(3), 764–769. <https://doi.org/10.1021/jf9903861>
- 499 Foubert, I., Fredrick, E., Vereecken, J., Sichien, M., & Dewettinck, K. (2008). Stop-and-return  
500 DSC method to study fat crystallization. *Thermochimica Acta*, 471(1–2), 7–13.  
501 <https://doi.org/10.1016/j.tca.2008.02.005>
- 502 Goibier, L., Pillement, C., Monteil, J., Faure, C., & Leal-Calderon, F. (2019). Emulsification of

503 non-aqueous foams stabilized by fat crystals: Towards novel air-in-oil-in-water food colloids.  
504 *Food Chemistry*, 293, 49–56. <https://doi.org/10.1016/j.foodchem.2019.04.080>

505 Hansen, T. B., Simone, E., Nagy, Z., & Qu, H. (2017). Process Analytical Tools to Control  
506 Polymorphism and Particle Size in Batch Crystallization Processes. *Organic Process  
507 Research and Development*, 21(6), 855–865. <https://doi.org/10.1021/acs.oprd.7b00087>

508 Heymans, R., Tavernier, I., Dewettinck, K., & Van der Meeren, P. (2017). Crystal stabilization of  
509 edible oil foams. *Trends in Food Science and Technology*, 69, 13–24.  
510 <https://doi.org/10.1016/j.tifs.2017.08.015>

511 Himawan, C., Starov, V. M., & Stapley, A. G. F. (2006). Thermodynamic and kinetic aspects of  
512 fat crystallization. *Advances in Colloid and Interface Science*, 122, 3–33.  
513 <https://doi.org/10.1016/j.cis.2006.06.016>

514 Hussin, A. B. B. H., & Povey, M. J. W. (1984). A study of dilatation and acoustic propagation in  
515 solidifying fats and oils: II. Experimental. *Journal of the American Oil Chemists' Society*,  
516 61(3), 560–564. <https://doi.org/10.1007/BF02677032>

517 Jaeggi, A., Rajagopalan, A. K., Morari, M., & Mazzotti, M. (2021). Characterizing Ensembles of  
518 Platelike Particles via Machine Learning. *Industrial & Engineering Chemistry Research*,  
519 60(1), 473–483. <https://doi.org/10.1021/acs.iecr.0c04662>

520 Jose, J., & Netto, G. (2019). Role of solid lipid nanoparticles as photoprotective agents in  
521 cosmetics. *Journal of Cosmetic Dermatology*, 18(1), 315–321.  
522 <https://doi.org/10.1111/jocd.12504>

523 Kloek, W., Walstra, P., & Van Vliet, T. (2000). Nucleation kinetics of emulsified triglyceride

524 mixtures. *JAOCS, Journal of the American Oil Chemists' Society*, 77(6), 643–652.  
525 <https://doi.org/10.1007/s11746-000-0104-7>

526 Ladd Parada, M., Povey, M. J., Vieira, J., Rappolt, M., & Ries, M. E. (2019). Early stages of fat  
527 crystallisation evaluated by low-field NMR and small-angle X-ray scattering. *Magnetic  
528 Resonance in Chemistry*, 57(9), 686–694. <https://doi.org/10.1002/mrc.4860>

529 Lipp, M., Simoneau, C., Ulberth, F., Anklam, E., Crews, C., Brereton, P., ... Wiedmaier, C.  
530 (2001). Composition of genuine cocoa butter and cocoa butter equivalents. *Journal of Food  
531 Composition and Analysis*, 14(4), 399–408. <https://doi.org/10.1006/jfca.2000.0984>

532 Martini, S., Bertoli, C., Herrera, M. L., Neeson, I., & Marangoni, A. (2005a). Attenuation of  
533 ultrasonic waves: Influence of microstructure and solid fat content. *JAOCS, Journal of the  
534 American Oil Chemists' Society*, 82(5), 319–328. [https://doi.org/10.1007/s11746-005-1073-  
535 6](https://doi.org/10.1007/s11746-005-1073-6)

536 Martini, S., Bertoli, C., Herrera, M. L., Neeson, I., & Marangoni, A. (2005b). In situ monitoring  
537 of solid fat content by means of pulsed nuclear magnetic resonance spectrometry and  
538 ultrasonics. *JAOCS, Journal of the American Oil Chemists' Society*, 82(5), 305–312.  
539 <https://doi.org/10.1007/s11746-005-1071-8>

540 McClements, D. J. J., & Povey, M. J. W. (1988). Ultrasonic velocity measurements in some liquid  
541 triglycerides and vegetable oils. *Journal of the American Oil Chemists' Society*, 65(11),  
542 1787–1790. <https://doi.org/10.1007/BF02542383>

543 McClements, D. J., & Povey, M. J. W. (1992). Ultrasonic analysis of edible fats and oils.  
544 *Ultrasonics*, 30(6), 383–388. [https://doi.org/10.1016/0041-624X\(92\)90094-3](https://doi.org/10.1016/0041-624X(92)90094-3)

545 McCLEMENTS, D. J., & POVEY, M. J. W. (1987). Solid fat content determination using  
546 ultrasonic velocity measurements. *International Journal of Food Science & Technology*,  
547 22(5), 491–499. <https://doi.org/10.1111/j.1365-2621.1987.tb00514.x>

548 Metilli, L., Lazidis, A., Francis, M., Marty-Terrade, S., Ray, J., & Simone, E. (2021). The Effect  
549 of Crystallization Conditions on the Structural Properties of Oleofoams Made of Cocoa Butter  
550 Crystals and High Oleic Sunflower Oil. *Crystal Growth & Design*, 21(3), 1562–1575.  
551 <https://doi.org/10.1021/acs.cgd.0c01361>

552 Miles, C. A., Fursey, G. A. J., & Jones, R. C. D. (1985). Ultrasonic estimation of solid/liquid ratios  
553 in fats, oils and adipose tissue. *Journal of the Science of Food and Agriculture*, 36(3), 215–  
554 228. <https://doi.org/10.1002/jsfa.2740360312>

555 Morris, L., Simone, E., Glover, Z. J., Powell, H., Marty-Terrade, S., Francis, M., & Povey, M. J.  
556 (2021). Dynamic monitoring of glycine crystallisation with low power ultrasound reflection  
557 spectroscopy. *Chemical Engineering Research and Design*, 170, 213–223.  
558 <https://doi.org/10.1016/j.cherd.2021.04.003>

559 Ochsenein, D. R., Vetter, T., Schorsch, S., Morari, M., & Mazzotti, M. (2015). Agglomeration  
560 of needle-like crystals in suspension: I. Measurements. *Crystal Growth and Design*, 15(4),  
561 1923–1933. <https://doi.org/10.1021/acs.cgd.5b00094>

562 Patel, A. R., & Dewettinck, K. (2016). Edible oil structuring: An overview and recent updates.  
563 *Food and Function*, 7(1), 20–29. <https://doi.org/10.1039/c5fo01006c>

564 Patel, D., Patel, B., & Thakkar, H. (2021). Lipid Based Nanocarriers: Promising Drug Delivery  
565 System for Topical Application. *European Journal of Lipid Science and Technology*, 123(5),

566 1–12. <https://doi.org/10.1002/ejlt.202000264>

567 Povey, M. J. W. (2017). Applications of ultrasonics in food science - novel control of fat  
568 crystallization and structuring. *Current Opinion in Colloid and Interface Science*, 28, 1–6.  
569 <https://doi.org/10.1016/j.cocis.2016.12.001>

570 Pu, Y., Chaudhry, S., Parikh, M., & Berry, J. (2015). Drug Development and Industrial Pharmacy  
571 Application of in-line viscometer for in-process monitoring of microcrystalline cellulose-  
572 carboxymethylcellulose hydrogel formation during batch manufacturing Application of in-  
573 line viscometer for in-process monitoring of microcrystalline cellulose-  
574 carboxymethylcellulose hydrogel formation during batch manufacturing. *Drug Dev Ind*  
575 *Pharm*, 41(1), 28–34. <https://doi.org/10.3109/03639045.2013.845837>

576 Rathore, A. S., Bhambure, R., & Ghare, V. (2010). Process analytical technology (PAT) for  
577 biopharmaceutical products. *Analytical and Bioanalytical Chemistry*, 398(1), 137–154.  
578 <https://doi.org/10.1007/s00216-010-3781-x>

579 Rios, R. V., Pessanha, M. D. F., Almeida, P. F. de, Viana, C. L., & Lannes, S. C. da S. (2014).  
580 Application of fats in some food products. *Food Science and Technology (Campinas)*, 34(1),  
581 3–15. <https://doi.org/10.1590/s0101-20612014000100001>

582 Salami, H., McDonald, M. A., Bommarius, A. S., Rousseau, R. W., & Grover, M. A. (2021). In  
583 Situ Imaging Combined with Deep Learning for Crystallization Process Monitoring:  
584 Application to Cephalexin Production. *Organic Process Research and Development*, 25(7),  
585 1670–1679. <https://doi.org/10.1021/acs.oprd.1c00136>

586 Simone, E., Saleemi, A. N., & Nagy, Z. K. (2015). In situ monitoring of polymorphic

587 transformations using a composite sensor array of Raman, NIR, and ATR-UV/vis  
588 spectroscopy, FBRM, and PVM for an intelligent decision support system. *Organic Process*  
589 *Research and Development*, 19(1), 167–177. <https://doi.org/10.1021/op5000122>

590 Simone, Elena, Tyler, A. I. I., Kuah, D., Bao, X., Ries, M. E., & Baker, D. (2019). Optimal Design  
591 of Crystallization Processes for the Recovery of a Slow-Nucleating Sugar with a Complex  
592 Chemical Equilibrium in Aqueous Solution: The Case of Lactose. *Organic Process Research*  
593 *& Development*, 23(2), 220–233. <https://doi.org/10.1021/acs.oprd.8b00323>

594 Singh, A. P., McClements, D. J., & Marangoni, A. G. (2002). Comparison of ultrasonic and pulsed  
595 NMR techniques for determination of solid fat content. *JAOCS, Journal of the American Oil*  
596 *Chemists' Society*, 79(5), 431–437. <https://doi.org/10.1007/s11746-002-0501-y>

597 Singh, A. P., McClements, D. J., & Marangoni, A. G. (2004). Solid fat content determination by  
598 ultrasonic velocimetry. *Food Research International*, 37(6), 545–555.  
599 <https://doi.org/10.1016/j.foodres.2003.12.010>

600 Szilágyi, B., & Nagy, Z. K. (2018). Aspect Ratio Distribution and Chord Length Distribution  
601 Driven Modeling of Crystallization of Two-Dimensional Crystals for Real-Time Model-  
602 Based Applications. *Crystal Growth & Design*, 18(9), 5311–5321.  
603 <https://doi.org/10.1021/acs.cgd.8b00758>

604 Tang, D., & Marangoni, A. G. (2008). Fractal dimensions of simulated and real fat crystal networks  
605 in 3D space. *JAOCS, Journal of the American Oil Chemists' Society*, 85(6), 495–499.  
606 <https://doi.org/10.1007/s11746-008-1237-7>

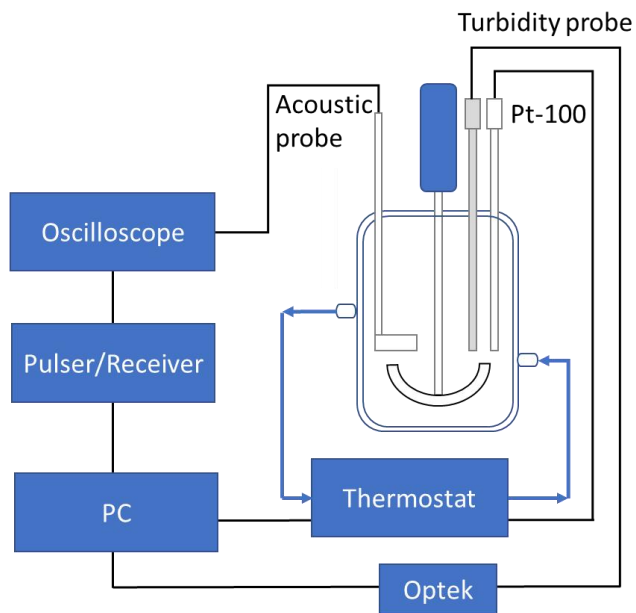
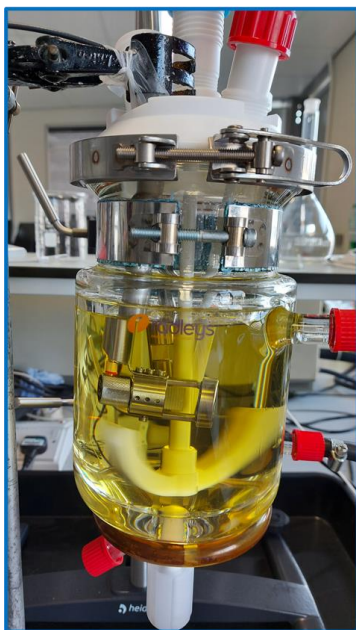
607 Titiz-Sargut, S., & Ulrich, J. (2003). Application of a protected ultrasound sensor for the

608 determination of the width of the metastable zone. *Chemical Engineering and Processing:*  
609 *Process Intensification*, 42(11), 841–846. [https://doi.org/10.1016/S0255-2701\(02\)00215-5](https://doi.org/10.1016/S0255-2701(02)00215-5)

610 Wasalathanthri, D. P., Rehmann, M. S., Song, Y., Gu, Y., Mi, L., Shao, C., ... Li, Z. J. (2020).  
611 Technology outlook for real-time quality attribute and process parameter monitoring in  
612 biopharmaceutical development—A review. *Biotechnology and Bioengineering*, 117(10),  
613 3182–3198. <https://doi.org/10.1002/bit.27461>

614

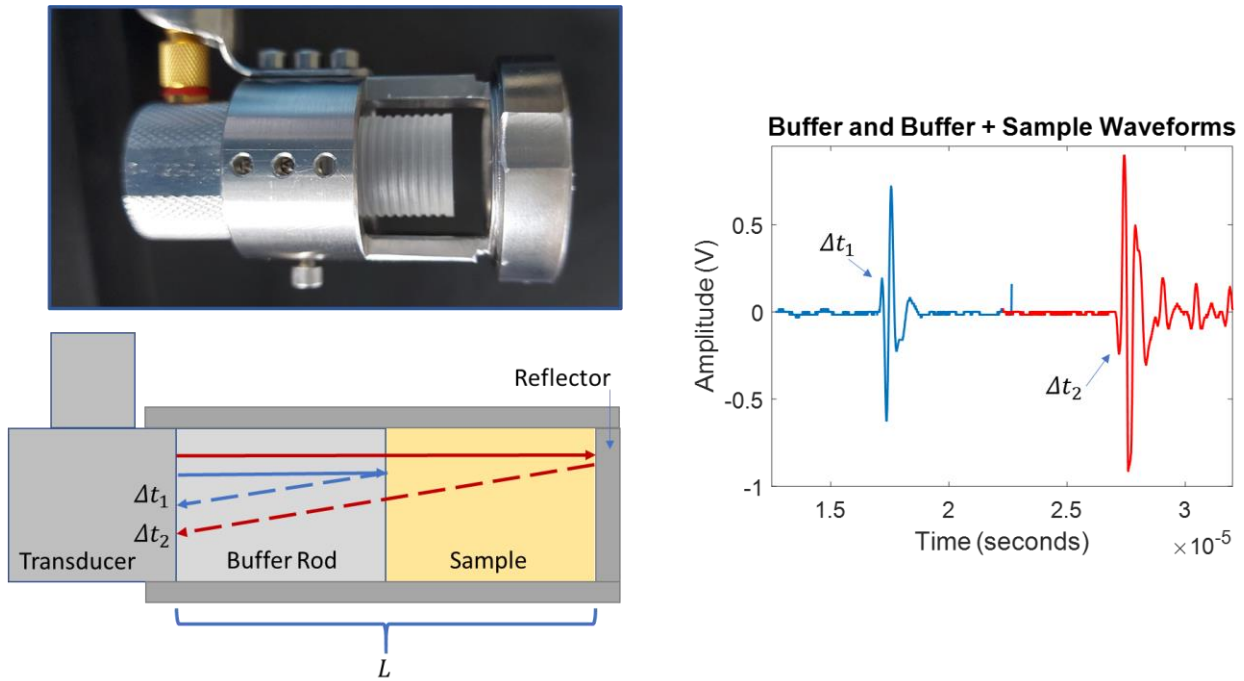
615



616

617 Figure 1. Crystallization vessel fitted with a Pt-100 thermocouple, the turbidity probe and the  
618 custom ultrasound probe (left), schematic depiction of the rig used in this paper (right).

619



620

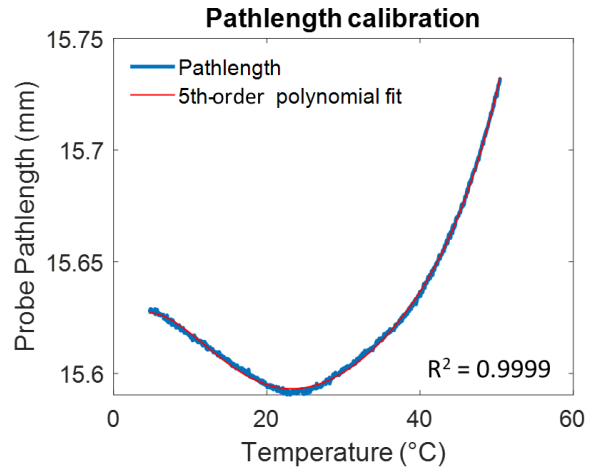
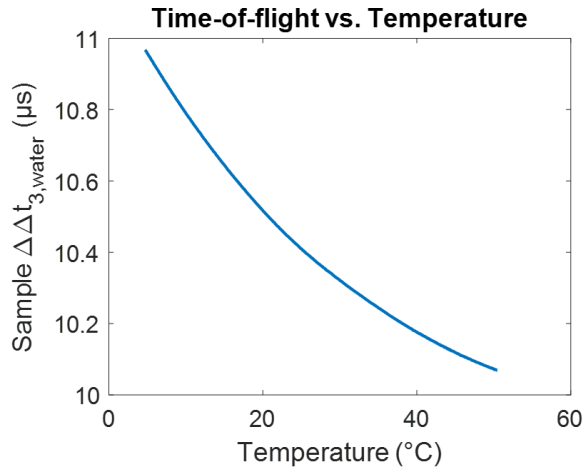
621 Figure 2. Schematic drawing of the custom acoustic probe (left) and the measured waveforms

622 obtained by the reflection of the buffer rod (blue), and buffer rod and sample (red) (right).  $\Delta t_1$  and

623  $\Delta t_2$  represent the time-of-flight of the pulse travelling through the buffer rod and through the buffer

624 rod and sample, respectively.

625

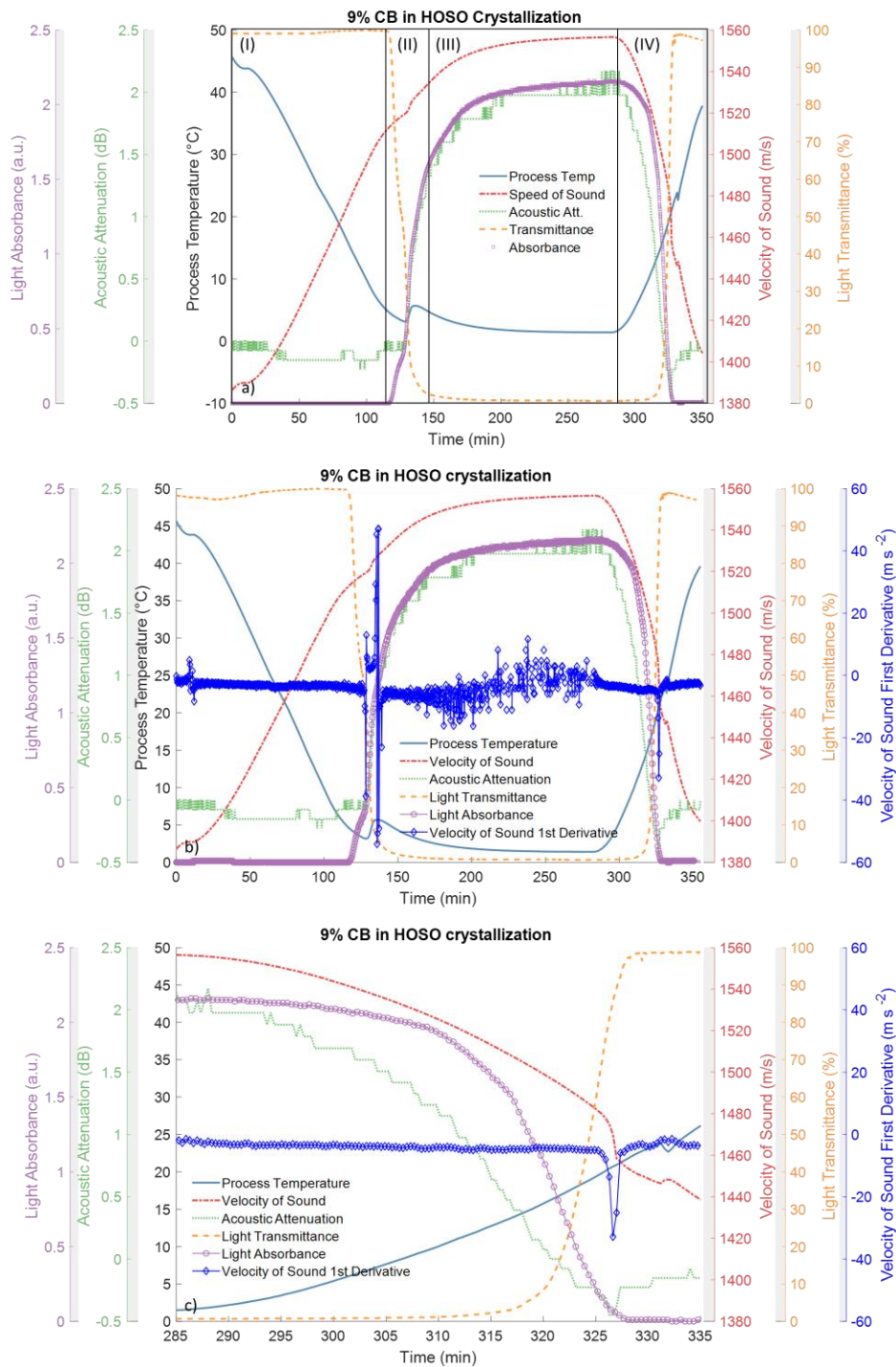


626

627 Figure 3. Measured time-of-flight in distilled water between 60 °C and 5 °C (left), calculated

628 sample path length and 5<sup>th</sup>-order polynomial fitting (right).

629

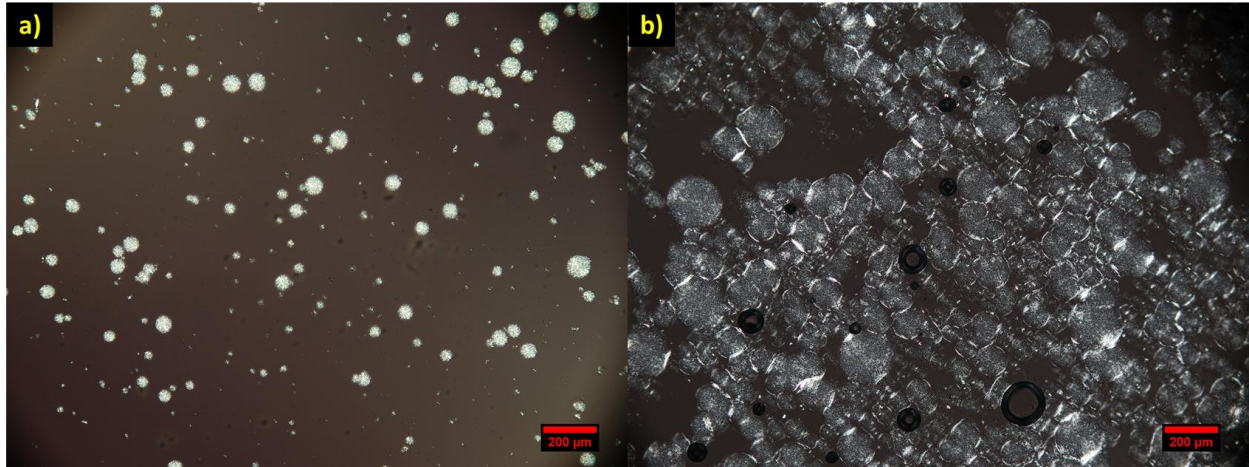


630

631 Figure 4. Process Analytical Technologies (PAT) tools plot of the crystallization of a 9% w/w CB  
 632 in HOSO blend (a). The different regions of the crystallization process are indicated with roman  
 633 numerals (I-IV). First derivative plot overlaid on the other PAT tools parameters (b) and

634 enlargement of the melting zone (c). Process temperature (-), velocity of sound (-·-), acoustic  
635 attenuation (··), light transmittance (--), light absorbance (-o-) and first derivative of the velocity  
636 of sound (-◇-) .

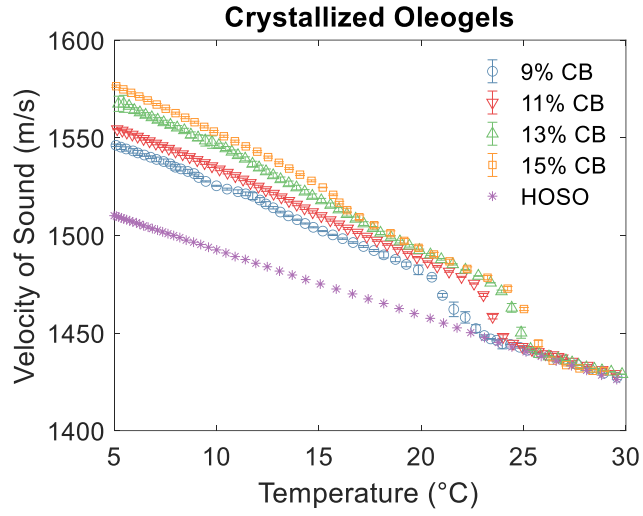
637



638

639 Figure 5. Polarized Light Microscopy (PLM) image of a 15% CB in HOSO mixture while  
640 crystallizing under shear. Onset of nucleation (a) and formation of the spherical aggregates  
641 network (b). Scale bar is 200  $\mu\text{m}$  for both figures.

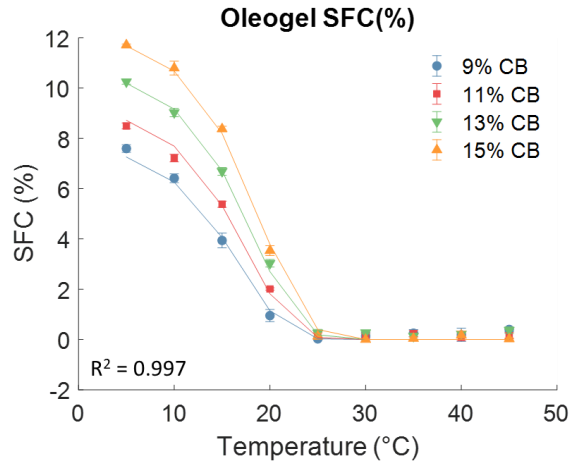
642



643

644 Figure 6. Velocity of sound of crystallized CB/HOSO oleogels between 5 and 35 °C. The error  
 645 bars show the standard deviation of three measurements for each concentration. The velocity of  
 646 sound of the pure HOSO phase is also plotted for reference.

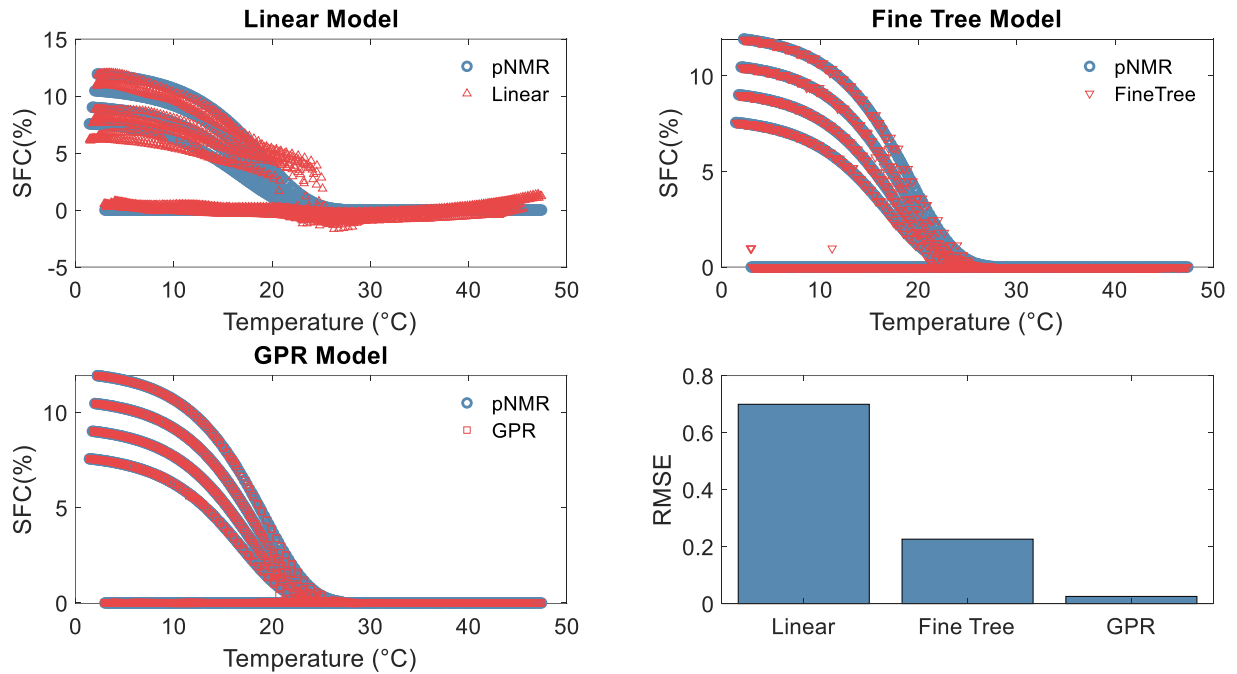
647



648

649 Figure 7. SFC% calculated with *p*NMR with respect to temperature for the different CB/HOSO  
 650 blends. The datapoints were fitted using a Gompertz-type model, similar to the one described in  
 651 Farmani (2015).

652



653

654 Figure 8. Predicted  $SFC(\%)$  values obtained from the supervised-machine learning models,

655 compared against the measured  $SFC(\%)$  from  $pNMR$  (blue circles) as a function of temperature:

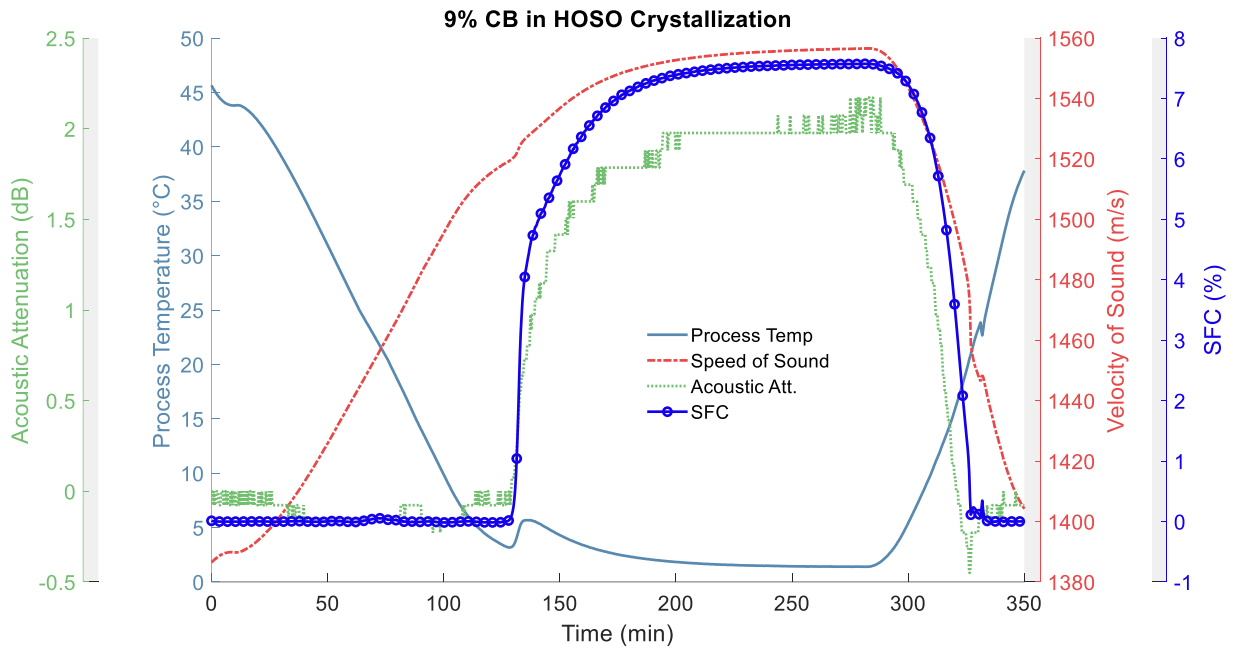
656 a) Linear model (red upward triangles), b) Fine Tree model (red downward triangles), c) Gaussian

657 Process Regression (red squares) and d) histogram plot showing the RMSE on the  $SFC(\%)$  values

658 for each model.

659

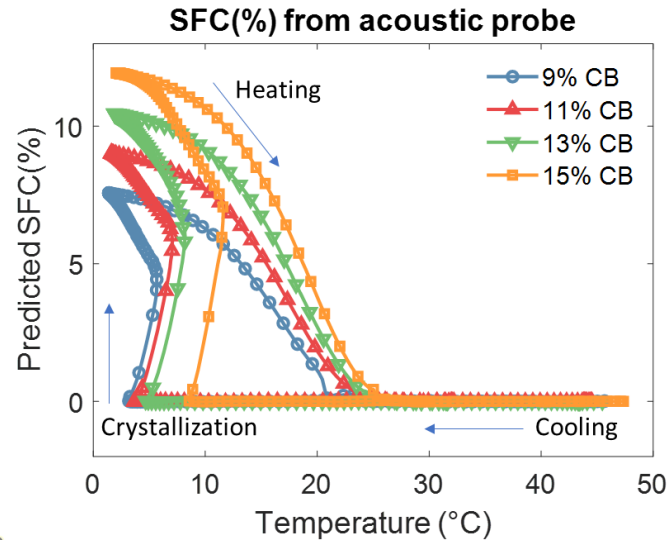
660



661

662 Figure 9. Process Analytical Technologies (PAT) tools plot of the crystallization of a 9% w/w CB  
663 in HOSO blend. Process temperature (-), velocity of sound (-·-), acoustic attenuation (···), and  
664 predicted SFC% with the GPR model (-o-).

665



666

667 Figure 10. Evolution of ultrasound predicted SFC% during cooling from 40 °C to 5 °C and heating

668 (5 °C to 40 °C) for oleogels containing different CB w/w %.

669

670

671 Table 1. Velocity of sound and acoustic attenuation for oleogel samples at the end of the  
672 crystallization (5 °C).

CB % (w/w)	Velocity of Sound (5 °C) (m/s)	Acoustic Attenuation (5 °C) (dB)
9	1545.8 ± 2.2	2.25 ± 1.13
11	1555.1 ± 1.2	2.42 ± 0.09
13	1567.5 ± 6.5	2.61 ± 0.06
15	1578.6 ± 4.0	3.31 ± 0.42

673

674