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

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| 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 oils (oleogels) has gained significant attention from academia and industry alike, due to the 32 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

material (Co & Marangoni, 2012; Heymans et al., 2017). The total amount of crystals, *i.e.* the solid
fat content (*SFC*%), is a general parameter strongly related to some macroscopic properties of fatbased materials, such as the melting point, hardness and texture (Himawan et al., 2006). Hence,
monitoring *SFC*% during crystallization processes is important to determine when equilibrium
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

are sparse examples of PAT tools applied to the manufacturing process of materials with similar
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_{sample}) is then calculated from the travelled path length as a function 75 of time and temperature. The acoustic attenuation (α), *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 crystallization were carried out in quiescent conditions, and/or with small sample volumes, thus 84

excluding the effect of shear and secondary nucleation on crystallization which are predominant
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).

The approach can be extended to the actual control of crystallization processing, for example through the use of convolutional neural network (CNN) feedback control to dissolve undesired precipitated impurities during the crystallization of active pharmaceutical ingredients (APIs) (Salami et al., 2021). Recently, ML has been applied to ultrasonic reflectance measurements to 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 (pNMR) 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 pNMR, 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**

Refined, bleached and deodorized cocoa butter (CB) and high-oleic sunflower oil (HOSO) were kindly provided by Nestlé PTC Confectionery (York, UK) and used without any further purification. CB was melted at 65 °C for one hour, and then mixed with HOSO at 9%, 11%, 13% and 15% concentration by weight. HOSO contains usually the following fatty acids (by weight): 86% oleic acid, 5% stearic acid, 3% linoleic acid, 3% palmitic acid, 1.5% behenic acid, and 0.7%
arachidic acid. CB normally contains by a weight about 26% palmitic acid, 36% stearic acid, 34%
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 of159 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 Δt_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 Δt_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 Δt_1 and Δt_2 were first determined with MATLAB from the original waveform, using the leading-167 168 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

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

$$\Delta \Delta t_3 = \Delta \Delta t_2 - \Delta \Delta t_1 \tag{Eq. 1}$$

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

$$c_{water} = 3.16 \cdot 10^{-9} T^5 - 1.48 \cdot 10^{-6} T^4 + 3.35 \cdot 10^{-4} T^3 - 5.81 \cdot 10^{-2} T^2$$

$$+ 5.04 \cdot T + 1.40 \cdot 10^3$$
(Eq. 2)

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

$$L_{calibrated} = \Delta \Delta t_{3,water} \times c_{water}$$
(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

the velocity of sound during the crystallization experiments with Equation 4

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

190 **2.3.2 Acoustic Attenuation**

191 The acoustic attenuation quantifies the acoustic power absorbed and scattered by the sample, and192 is affected by several factors, including the onset of phase transitions and scattering phenomena

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

$$\alpha = -20\log\frac{A}{A_0} \tag{Eq. 5}$$

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; the response matrix contained the SFC% values, measured with pNMR, between 5 and 45 °C for 206 207 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 all iterations is calculated as R², RMSE and other statistical parameters. In the discussion, the Root 222 223 Mean Square Error values (RMSE) were compared to select the most accurate predictive model.

In addition to the predictive model, an equation that estimates the equilibrium *SFC*% as function of the temperature and the concentration of CB in sunflower oil was determined using the *Curve Fitting Tool* in MATLAB2021a. A custom equation based on literature was used to fit the experimental data using the *NonlinearLeastSquares* method; R², RMSE and 95% confidence 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 Gompertz-type model (Farmani, 2015) to obtain the SFC(%) as a function of the temperature and 237 238 CB%.

9 **3.** Results and Discussion

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

The crystallization of one 9% CB w/w in HOSO sample, monitored with turbidity and the acoustic
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 (α or β) 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 α 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 β (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.

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

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

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

This set of experiments showed that the tested acoustic probe could be used to monitor crystallization processes, although it seemed less sensitive than light turbidimetry to the detection of early nucleated crystals, perhaps due to their liquid crystalline nature. Experiments carried out with a higher weight % of CB showed similar trends for the acoustic and
light signals. The velocity of sound of the crystallized oleogels at different % w/w of added CB,
between 5 and 35°C, is shown in Figure 6.

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

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

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

By inspecting the *SFC*% values at 5 °C, it can be noted that all samples displayed a lower *SFC*% compared to the total amount of added cocoa butter. This is because the main triacylglycerides species that are solid at ambient temperature, 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), 1,3distearoyl-2-oleoyl-glycerol (SOS) and 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS), constitute about 80% w/w of cocoa butter, while the remaining 20% comprises mono- and diglycerides, phospholipids, sterols and free fatty acids that are liquid at ambient conditions (Dimick, 1991). It is worth noticing that the *p*NMR measurements present an average standard deviation of 0.101%, which is a more than acceptable value for industrial applications (particularly the confectionary sector).

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

$$SFC(\%)(T,CB) = (b_0 + b_1CB)e^{-e^{\frac{-(T-(b_{01}+(b_{11}CB)))}{c}}}$$
(Eq. 6)

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

This fit enabled calculation of the equilibrium SFC(%) as a function of temperature and CB concentration, which can be used in combination with the predictive models determined in the next 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 .

By inspecting the cooling profile, it can be noted that crystal growth, signalled by the increase in SFC%, occurred at higher crystallization temperatures for oleogels containing higher % of CB, as also reported previously in Metilli et al. (2021) for this type of system. Whereas, by inspecting the heating profile, the *SFC*% decreased with the temperature with the same trend observed with *p*NMR. The estimated values of *SFC*% at 5 °C during heating were 7.3, 8.7, 10.2 and 11.7% for the 9, 11, 13 and 15% CB samples, respectively. These values are very close to the equilibrium SFC% measured with *p*NMR at the same temperature (7.6, 8.5, 10.3 and 11.7%) indicating the reliability of the predictive model built. Furthermore, both *p*NMR and acoustic spectroscopy showed agreement that at 25 °C all samples returned to the full liquid state.

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

388

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 pNMR. 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 pNMR. 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**.

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

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426 Author Contributions

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429

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442 ABBREVIATIONS

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

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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).



Figure 2. Schematic drawing of the custom acoustic probe (left) and the measured waveforms obtained by the reflection of the buffer rod (blue), and buffer rod and sample (red) (right). Δt_1 and Δt_2 represent the time-of-flight of the pulse travelling through the buffer rod and through the buffer rod and sample, respectively.



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

sample path length and 5th-order polynomial fitting (right).



Figure 4. Process Analytical Technologies (PAT) tools plot of the crystallization of a 9% w/w CB
in HOSO blend (a). The different regions of the crystallization process are indicated with roman
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 $(-\diamond)$.



Figure 5. Polarized Light Microscopy (PLM) image of a 15% CB in HOSO mixture while
crystallizing under shear. Onset of nucleation (a) and formation of the spherical aggregates
network (b). Scale bar is 200 μm for both figures.



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



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).



Figure 8. Predicted SFC(%) values obtained from the supervised-machine learning models, compared against the measured SFC(%) from *p*NMR (blue circles) as a function of temperature: a) Linear model (red upward triangles), b) Fine Tree model (red downward triangles), c) Gaussian Process Regression (red squares) and d) histogram plot showing the RMSE on the SFC(%) values for each model.



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



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 %.

| 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 |

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