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# Insight in ultrasonic shear reflection parameters by studying temperature and limonene influence on cocoa butter crystallization

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ABSTRACT

In this study an inverse model was developed to derive relevant ultrasonic parameters from ultrasonic shear reflectometry measurements. The inverse model includes four variable parameters: $t_{ind}$ (induction time), $K$ (crystallization rate), $v_{s2}$ (shear ultrasonic velocity) and $a_{s2}$ (shear ultrasonic attenuation coefficient). Both the temperature effect and the effect of a minor component limonene (in different concentrations) on the isothermal crystallization of cocoa butter were studied with the ultrasonic shear reflectometry technique and associated inverse model. Subsequently, the ultrasonic parameters were compared with results of conventional techniques to monitor fat crystallization (DSC, PLM). The study shows that $t_{ind}$ and $K$ provide information on the kinetics of the microstructure development. The parameter $v_{s2}$ is related with the equilibrium SFC, while $a_{s2}$ is both influenced by the SFC and the organization of the crystals in the network, yielding information about the microstructure of the crystallized samples.

*Industrial relevance:* The microstructure of crystallized fat determines to a large extent the macroscopic properties of fat rich products, such as texture, mouthfeel,… Hence, monitoring the crystallization behavior (including not only the primary crystallization but also the microstructural development) during the production process is of utmost importance in order to obtain high quality end products. The ultrasonic shear reflectometry technique is a fast non-destructive technique which provides quantitative data about the crystallization process based on an inverse model. The simplicity of the technique offers potential for inline control. This may be beneficial to evaluate the crystallization process under different process conditions and to stimulate product innovation as more insight can be obtained in the microstructure development of new products.



## 1. Introduction

As the fat crystallization process determines to a large extent the quality of fat containing food products, several methodologies have been proposed to monitor the fat crystallization process. These techniques differ in the fat crystallization features they measure, and monitor various aspects of the fat crystallization process on multiple length scales.

Different structural levels can be distinguished in a fat crystal network. The crystallization process starts with the primary crystallization whereby the triacylglycerols (TAG) form crystalline lamellae. As the fatty acid chains of the TAG can adopt different molecular arrangements, distinct packing modes arise which result in different polymorphic forms with specific physical properties. The crystalline lamellae stack in crystalline nanoplatelets with dimensions in the hundred-nanometres range (Marangoni et al., 2012). The nanoplatelets then aggregate further through van der Waals attraction and clot to form larger clusters which interact even further, finally resulting in the formation of a continuous three-dimensional fat crystal network. The number, size, shape and spatial distribution of the particles and clusters of sizes between 1 and 200 μm define the microstructure, which is known to have an enormous influence on the macroscopic properties of fat products (Acevedo, Peyronel, & Marangoni, 2011; Narine & Marangoni, 1999).

The nanoscale structures can be investigated by X-ray diffraction (XRD): the different polymorphic forms have characteristic wide angle XRD patterns, while small angle XRD patterns can be used to determine the thickness of the individual crystalline lamella and even the crystalline domains (which correspond to the thickness of the nanoplatelets) via Scherrer analysis (Acevedo et al., 2011). As an alternative, various types of microscopy can be applied to visualize the different structure levels. Cryogenic transmission electron microscopy has been used to expose the nanoscale structures, whereas polarized light microscopy (PLM) is often used to display the microstructural level (Acevedo

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et al., 2011; Marangoni et al., 2012). Although very valuable information can be obtained with microscopy, the technique also has some drawbacks. The sample material between the glass slides must be sufficiently thin in order to limit the attenuation of the transmitted light. This leads to growth restricted to one dimension (Ghotra, Dyal, & Narine, 2002). In addition, quantitative data cannot be directly obtained from microscopy pictures.

Besides the structural rearrangements during crystallization, other properties of this liquid to solid transition can be probed. For instance, the relative amount of solid substance, better known as the solid fat content (SFC), can be determined by pulsed nuclear magnetic resonance (pNMR). As crystallization is an exothermic reaction, the released heat can also be used as a measure for the amount of crystallized material. This principle is applied when using differential scanning calorimetry (DSC) to monitor fat crystallization (Foubert, Dewettinck, & Vanrolleghem, 2003). Finally, the crystallization process can also be followed by changes in the viscoelastic properties revealing the fluid and/or solid character of the sample system. This can be done using oscillatory rheology (De Graef, Dewettinck, Verbeken, & Foubert, 2006) or using a viscometer (Dhonsi & Stapley, 2006).

The different techniques discussed above are the conventional techniques used to monitor fat crystallization processes, each with their advantages and disadvantages, but with the common limitation that they cannot be used for inline monitoring and control. Inline methods to monitor fat crystallization could have significant economic benefits, and therefore, there is a constant search for alternative, non-destructive techniques that can be used for inline inspection. Furthermore, the crystallization depends on the process conditions (temperature, shear, time) applied (Afoakwa, Paterson, Fowler, & Vieira, 2008) and it is therefore critical to monitor the crystallization process under the same process conditions, what is done automatically with an inline technique. NMR-MOUSE (Nuclear Magnetic Resonance—mobile universal surface explorer), Laser backscattering, Near-Infrared (NIR) spectroscopy and ultrasonic techniques have been suggested. NMR-MOUSE can be utilized for inline SFC measurements, but the technique is limited to static conditions and is less accurate than ultrasonics (Martini, Herrera, & Marangoni, 2005c). NIR spectroscopy allows to obtain microstructural information related to the size, shape and quantity of crystals via correlations between NIR spectra and measurements of viscosity and crystal content. Although these correlations are derived statistically and the relationship with the crystallization process is unclear (Bolliger, Zeng, & Windhab, 1999), Svenstrup, Heimdal, and Norgaard (2005) demonstrated that NIR spectroscopy could distinguish between different tempering procedures. Furthermore, NIR spectroscopy has also been applied for the on-line determination of the fat content of meat (Wu & Sun, 2013). Laser backscattering can be applied to monitor changes in particle size distribution during crystallization, and although the total particle count correlates well with the amount of crystallized fat, it does not measure the solid fat content in a direct way and merely serves as an estimation (Hishamuddin, Stapley, & Nagy, 2011).

Ultrasonic inspection is the most frequently studied non-destructive technique, because it is highly suitable for inline monitoring: capable of rapid and precise measurements, relatively inexpensive, non-hazardous, fully automatable and usable under stirring conditions (Garbolino, Ziegler, & Coupland, 2000; Martini, Bertoli, Herrera, Neeson, & Marangoni, 2005a; McClements & Povey, 1992). For clarity, low intensity ultrasound is used which does not have any effect on the food product, in contrast to the high power ultrasound which is used to alter food properties or facilitate production processes (Patist & Bates, 2008). A selection of measurement setups for ultrasonic crystallization monitoring have been described by Rigolle et al. (2015). Commonly, most research studies deal with longitudinal or pressure waves whereby particles move in the same direction as the propagated wave. The (reflection and) transmission of pressure waves can be used to determine the SFC as this property is correlated with the ultrasonic velocity. A disadvantage of this methodology is that it is limited to low SFC levels or thin materials due to the high attenuation of crystallized fat (Singh, McClements, & Marangoni, 2004). Nonetheless, data on attenuation can provide additional information about the polymorph (Häupler, Peyronel, Neeson, Weiss, & Marangoni, 2014) or microstructure (Martini, Bertoli, Herrera, Neeson, & Marangoni, 2005b).

In Rigolle et al. (2015), we developed an ultrasonic shear reflection technique to monitor fat crystallization. The main advantage of applying a reflection technique is that no problems with excessive attenuation of the fat arise. Furthermore, shear or transverse waves, where the movement of the particles is perpendicular to the direction of the propagating wave (McClements, 1997), seem more sensitive to changes in microstructure than pressure waves, as the former require a medium that displays shear elasticity (Létang, Piau, Verdier, & Lefebvre, 2001) to be propagated. In Rigolle et al. (2015) the shear reflectometry technique has been introduced together with an interpretation of the experimental results. In the present article, an inverse model is developed to derive quantitative parameters from the experimental results. Subsequently, these ultrasonically obtained parameters are compared with data from conventional techniques, such as DSC and PLM, to shed light on the interpretation of these parameters. To obtain relevant data for model validation, the crystallization process was modified in two ways: by changing the crystallization temperature and by adding a minor component limonene. Limonene is a diterpene, which is naturally present in the D-form in ethereal oils of lemon and orange and is relevant in a reduced fat chocolate context (Beckett, 2001; Do, Vieira, Hargreaves, Wolf, & Mitchell, 2008). The fat content affects the rheological and textural properties of chocolate (Afoakwa et al., 2008) and cannot be reduced without any effect on quality. Therefore, a viscosity adjusting component should be added to reduced-fat chocolate and a US Patent by Beckett (2001) states that adding limonene (in a concentration of up to 5% by weight) to a reduced-fat chocolate results in a lower viscosity and a softer chocolate, that melts more easily in the mouth, compared with the original reduced-fat chocolate without limonene. However, limonene drastically modifies the crystallization process by lowering the SFC, accelerating polymorphic transitions and changing the microstructure development (Do et al., 2008; Miyasaki et al., 2015; Ray, MacNaughtan, Chong, Vieira, & Wolf, 2011).

## 2. Materials and methods

### 2.1. Materials

The used cocoa butter was a standard factory product of West-African origin kindly provided by Barry Callebaut (Wieze, Belgium). D-Limonene (97%) was purchased from Sigma-Aldrich and was added to the melted cocoa butter at 85 °C in concentrations of 1%; 2.5%; 5% and 7.5% by weight. The samples were then stirred for 10 min with a magnetic stir plate to obtain a homogeneous mixture.

### 2.2. Ultrasonic shear reflectometry measurements

#### 2.2.1. Experiments

The experimental setup for the shear reflectometry measurements is extensively described in Rigolle et al. (2015). In brief, a shear wave transducer (V154-RB, 12.7 mm active diameter, 2.25 MHz central frequency, Olympus Corporation, Tokyo, Japan) is attached to the bottom side of a plexiglass plate, above which an aluminum sample holder (60 mm diameter) is placed. Liquid cocoa butter (20 g) at 30 °C, with or without limonene, is poured into the sample holder to form a layer of 7.7 to 8 mm. The sample holder is surrounded by a container filled with water, which is kept at constant temperature by refreshing it, using a pumping system (Masterflex L/S, Metrohm, Belgium), with water from a cryostat (RC6 LAUDA, Lauda-Königshofen, Germany) at a fixed temperature. The experiments were carried out in a temperature controlled room, and the temperature of the water bath and of the fat