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Free fatty acids and their esters modulate isothermal crystallization of anhydrous milk fat

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ABSTRACT

The effect of free fatty acids with different chain lengths or unsaturation degree on anhydrous milk fat (AMF) crystallization was evaluated. The impact of esterification was also studied using three triglycerides. Melted blends containing the additives at concentrations lower than 12 wt.% were quenched at 25 °C and isothermal crystallization was monitored by pulsed low-resolution nuclear magnetic resonance. In parallel, polarized light microscopy was used to observe the microstructure. Compounds based on long chain saturated fatty acids, *i.e.* palmitic, stearic, eicosanoic acids, tripalmitin and tristearin accelerated crystallization. Conversely, propanoic, hexanoic and oleic acids slowed down the process, while triacetin had no impact. Interestingly, above a critical concentration, the addition of palmitic, stearic or eicosanoic acids caused a transition from a one-step to two-step process. Gompertz model was used to fit the experimental data and to assess the influence of the molecular properties of the additives on the kinetic parameters.

1. Introduction

Common edible fats and oils are largely composed of triacylglycerols (TAGs) whose composition and structure determine their physical properties. It is well known that solid state fats may display several crystalline forms. In various food products, the fat phase is found, at least in part, in a crystallized form at the operating temperature or under storage conditions. The polymorphic state may influence the rheological properties or the stability of the products. For instance, fat crystallization in the β' form improves the spreadability of margarines and in-mouth sensory attributes like smoothness. Appropriate tempering of chocolate allows the desired melting texture and prevents fat blooming responsible for surface bleaching. Crystallization of TAGs is commonly described as a two-stage mechanism: nucleation and crystalline growth (Metin & Hartel, 2005). Nucleation can be classified into primary nucleation, when the melt is initially devoid of crystals, and secondary nucleation, when some crystals are already present to induce crystallization. Primary nucleation can occur through a heterogeneous mechanism in the presence of impurities or interfaces that elicit the process, or through a homogeneous pathway, based on the spontaneous formation of crystalline germs in bulk. Nucleation occurs when the temperature of a molten fat decreases under the temperature of fusion of at least one of its components. At a high cooling rate, the liquid phase may transiently remain in a metastable undercooled state, and the involved components become supersaturated (Marangoni & Wesdorp, 2013). This phenomenon reflects the existence of an energy barrier due to surface tension between the crystals and the liquid phase. Molecules start to aggregate in clusters that need to reach a critical size to further grow. After formation of large enough nuclei, the crystalline growth proceeds by accretion of molecules from the melt at the interfacial level. The growth can happen in several dimensions and depends on the structure of the crystal facets. It can be kinetically limited by the diffusion of molecules in the liquid phase and/or by their adsorption at the solid/liquid interface.

Although TAGs are the major compounds of fats, minor lipids such as free fatty acids (FFAs), monoacylglycerols (MAGs), diacylglycerols (DAGs) and phospholipids can influence the nucleation stage, the crystal growth and/or the polymorphic behavior

Abbreviations: AMF, anhydrous milk fat; DAG, diacylglycerol; FFA, free fatty acid; MAG, monoacylglycerol; p-NMR, pulsed nuclear magnetic resonance; SFC, solid fat content; TAG, triacylglycerol.

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(Smith, Bhaggan, Talbot, & Malssen, 2011; Bayés-García et al., 2015; Patel & Dewettinck, 2015; Ribeiro et al., 2015; Sato, Bayés-García, Calvet, Cuevas-Diarte, & Ueno, 2013; Rønholt, Mortensen, & Knudsen, 2013). Their effect depends on their nature, concentration and the processing conditions, such as the crystallization temperature (Foubert, Vanhoutte, & Dewettinck, 2004), the cooling rate (Ollivon, Relkin, Michon, Kalnin, & Mariette, 2005) and the shear rate (Kaufmann, Andersen, & Wiking, 2012).

Among the different fats studied, anhydrous milk fat (AMF) has generated considerable interest because of its wide use in food products. However, due to its complex TAG composition, the interactions between AMF and other non TAG-lipids are poorly understood. Several studies showed that removing minor compounds from AMF changed its kinetics of crystallization (Mazzanti, Guthrie, Sirota, Marangoni, & Idziak, 2004; Herrera, de León Gatti, & Hartel, 1999; Vanhoutte, 2003; Wright, Hartel, Narine, & Marangoni, 2000). However, the results are somehow controversial. Removing polar components of AMF could either enhance (Mazzanti et al., 2004; Vanhoutte, 2003; Wright et al., 2000) or slow down (Herrera et al., 1999) AMF crystallization. Such contradictory results could be due to compositional differences. More specifically, phospholipids were shown to delay the onset time of AMF isothermal crystallization (Vanhoutte, Dewettinck, Foubert, Vanlerberghe, & Huyghebaert, 2002; Vanhoutte, Foubert, Duplacie, Huyghebaert, & Dewettinck, 2002). Addition of DAG to AMF slowed down crystallization (Wright & Marangoni, 2002; Wright et al., 2000) without modifying the fat microstructure (Wright & Marangoni, 2003). In contrast, at 25 °C, AMF crystallization was accelerated by diolein and delayed by distearin (Foubert et al., 2004). Addition of a blend of MAG and DAG to AMF favored crystallization, that occurred at a higher temperature than in the absence of these partial glycerides (Foubert et al., 2004; Ollivon et al., 2005; Wright & Marangoni, 2002; Wright et al., 2000, 2003). Changing the temperature and concentration conditions of mono-olein or mono-stearin reversed their impact on AMF crystallization rate. Depending on those factors, mono-olein and mono-stearin could either accelerate or delay AMF crystallization (Foubert et al., 2004).

Only few studied have focused on the effect of free fatty acids on fat crystallization. Lauric, palmitic and oleic acids at concentrations from 2.5 to 5% delayed crystallization of purified coconut oil at 15 °C (Gordon & Rahman, 1991). The same effect on coconut oil was observed upon addition of lauric acid at 15% (Chaleepa, Szepes, & Ulrich, 2010; Gordon & Rahman, 1991). On the contrary, lauric acid increased the crystallization rate of trilaurin (Smith, Cebula, & Povey, 1994). The addition of stearic acid in dark chocolate did not affect the early crystallization of trisaturated TAGs, but slowed down the second step of cocoa butter crystallization (Loisel, Lecq, Keller, & Ollivon, 1998). In palm oil, it was observed that the final solid fat content decreased proportionally to FFA produced by lipolysis (Jacobsberg & Ho, 1976).

Despite the numerous studies, to the best of our knowledge, no data is available on the impact of FFAs on AMF crystallization. In this context, the main objective of this work was to study the effect of FFAs and of some of their derivatives on AMF crystallization. Molecules with different polarities and concentrations were probed: FFAs with various chain-lengths (propanoic, hexanoic, palmitic, stearic, oleic and eicosanoic acids), a monoglyceride, and homogeneous triacylglycerols (triacetin, tripalmitin and tristearin). Their effect on isothermal crystallization of AMF at 25 °C was investigated by pulsed low-field nuclear magnetic resonance (p-NMR). Gompertz model (Gompertz, 1825) was used to characterize the kinetic evolution of the solid fat content (SFC), with the aim of identifying the impact of relevant molecular parameters (chain length, unsaturation degree, esterification degree) on AMF crystallization kinetics. Potential mechanisms of action of additives were confronted to polarized light microscopic observations.

2. Materials and methods

2.1. Materials

AMF was supplied by Corman SA (Goé, Belgium) and used without further purification. AMF fatty acid composition was determined by gas chromatography. It was composed of approximately 6% short chain fatty acids (strictly less than 8 carbons), 20% midsize chains and 72% long chains (strictly more than 14 carbons), including 42% saturated 16 and 18 chains. Unsaturated chains represented 27% of the total fatty acids, oleic acid being the most abundant. The following FFAs and fatty acid esters were purchased from Sigma Aldrich (Saint-Quentin Fallavier, France): tripalmitin (purity >85%, M = 807 g/mol), tristearin (purity ≈ 99%, M = 891 g/mol), triacetin (purity ≈ 99%, M = 218 g/mol), eicosanoic acid (20:0, purity ≈ 99%, M = 312.5 g/mol), stearic acid (18:0, purity >95%, M = 284 g/mol), palmitic acid (16:0, purity ≈ 98%, M = 256 g/mol), hexanoic acid (6:0, purity >97%, M = 116 g/mol), propanoic acid (3:0, purity ≈ 99.5%, M = 74 g/mol), and oleic acid (18:1, purity >95%, M = 282 g/mol).

2.2. Isothermal crystallization

Blends of AMF and FFAs or fatty acid esters (from 1 to 11.6 wt.%) were prepared directly into standard 10-mm NMR tubes. Blends were heated at 85 °C until fully melted. The tubes were then vortexed to ensure homogenization and placed in a 80 °C water bath for 30 min before measurements. Isothermal crystallization of pure AMF and the various blends was studied at 25 °C by a low field p-NMR unit equipped with a temperature-controlled measuring probe (Minispec mq20, Bruker, Karlsruhe, Germany). The samples initially warmed at 80 °C were introduced into the measuring probe at the temperature set point of 25 °C. Crystallization took place directly in the NMR device and the SFC was continuously recorded at regular time intervals. Hereafter, each point in the SFC *vs.* time plots corresponds to the average of 4 scans. The sample temperature was continuously measured during the crystallization process using a nonmetallic optical temperature probe (FISO Inc, Quebec, Canada). To check the repeatability of the measurements, plain AMF was analyzed in triplicate. Differences between the three analyses were always lower than 0.3 SFC units.

2.3. Modeling of crystallization SFC curves

To describe the crystallization behavior quantitatively, the experimental SFC *vs.* time plots were analyzed by means of Avrami and modified Gompertz models. The Avrami model considers both the nucleation and crystal growth stages (Marangoni, 1998). It is based on several assumptions: isothermal transformation, spatially random nucleation, linear growth kinetics depending only on the temperature (not on time), and constant density of the growing crystals (Marangoni & Wesdorp, 2013). Within this model, the kinetic evolution of the SFC is given by Eq. (1):

$$SFC(t) = a \cdot \exp(-kt^m) \quad (1)$$

where a is the final SFC (asymptotic value) and k is the crystallization rate. The exponent m, referred to as the crystallization index, depends on the nucleation type (instantaneous or sporadic) and on the spatial dimension of growth (as needles, disks or spheres).

The use of Gompertz model for lipids is based on the analogy between fat crystallization and microbial growth (Kloek, 1998). Gompertz equation is empirical: it does not provide a mechanical