Systematic investigation of lard polymorphism using combined DSC and time-resolved synchrotron X-ray diffraction

The polymorphic behavior of lard was systematically investigated by differential scanning calorimetry (DSC) while simultaneously monitoring the formation of the different crystal forms with X-ray diffraction (XRDT). To interpret the complex polymorphic evolution of the sample analyzed by regular DSC, both XRD patterns and DSC curves were recorded at the same time from the same sample (20 mg) using a laboratory-made calorimeter (http://www.umr-cnrs8612.u-psud.fr/Francais/pdf/MICROCALIX.pdf) installed at a synchrotron radiation bench capable of both small- and wide-angle X-ray diffraction and time-resolved experiments. Lard exhibits a large melting range ($230\text{ C}/C20$) with three main crystallization peaks. In this range, lard polymorphism was investigated by varying the cooling and heating rates between 10 and 0.15 K/min, and by isothermal recrystallization at $210$ and $15\text{ C}$. XRD lines observed by SAXS at about 35, 43.8 and 48.2 Å are identified to subcells $a$, $b'1$, $b'2$ and $b$, and attributed to the main DSC peaks. The crystalline forms appearing during the cooling depend on the cooling rate and on the final temperature. Higher cooling rates lead to unstable $a$ forms that transform subsequently upon heating and/or storage into $b'$ forms. The persistence of a monotropic transformation at $210\text{ C}$ that was monitored during isothermal storage is explained by the presence of a liquid phase at this temperature.

Keywords: Crystallization kinetics, DSC/XRDT, lard polymorphism, phase transition, storage conditions, structure, synchrotron radiation, TAG.

1 Introduction

Meat production from pigs was estimated at about 93 MT in 2001, from which about 50% and 22% were produced in China and Europe, respectively. Pork represents almost 40% of the world-wide daily meat protein intake. Lard is the fat obtained by rendering fatty tissue of the hog, the domestic pig. Natural lard has a characteristic waxy texture and exhibits unsatisfying bakery qualities, which are frequently corrected by fat blending or interesterification in making commercial shortenings [1–4]. Recently published studies concerning lard focus mostly on composition and analysis of genuine or modified lard [5–10]. The composition of lard varies with the hog's food [11–13] and mainly comprises a few long-chain major fatty acids which are about C16 24%, C18 14%, C18:1 41%, C18:2 10% [13]. Contrary to what is found for other fats, oleic acid is mostly found in the 1- and 3-position of glycerol, while palmitic acid is mostly esterified in the 2-position. Then, the 2-palmitooleostearin (PSO) and 2-palmitodiolein (OPO) are the major triacylglycerols (TAG) of lard, with about 13% and 19%, respectively [13]. The amount of the major trisaturated triacylglycerol, 2-palmitodistearin (SPS), is highly variable (2–10%). Although composed of only a few TAG, lard, as many other fats, exhibits complex thermal properties [6–10, 14, 15]. Indeed, when differential scanning calorimetry (DSC) is used for the investigation of the thermal properties of fats, often several peaks are observed on heating and cooling of samples. These DSC peaks reflect the occurrence of numerous thermal transitions, the temperatures and enthalpies of which vary as a function of sample thermal history [16–19]. This complexity, which is also related to the fat composition, is drastically enhanced by the existence of a polymorphism of monotropic type for each single TAG [18, 20–24]. For instance, up to six crystalline forms have been evidenced for monounsaturated triacylglycerols such as POP [23–26]. However, the identification of the polymorphic forms of pure TAG does not allow the straightforward interpretation of complex DSC recordings [2, 5–
effects of cooling and heating rates on the formation of the various crystalline lard polymorphism species were studied in the range of 0.15–10 K/min. Phase transitions were also observed during isothermal conditioning at low temperatures.

2 Materials and methods

2.1 Lard sample preparation

Lard was provided by Lusissier Bordeau Chesnel (Changé, Champagne; France). Samples were heated to 70 °C, homogenized and divided into aliquots of smaller quantities to be analyzed as received. When not specified differently, the thermal history of all samples was systematically annihilated by heating them for about 10 min to at least 10 K above their final melting point (≥70 °C).

2.2 DSC

Thermal analysis was performed using a DSC 7 (Perkin Elmer) equipped with a cooling device (Intracooler II) supported by Pyris Thermal Analyzing Systems version 3.52. Recordings were obtained by measuring the heat transfer either by cooling or heating at constant rates or in isothermal mode. All samples, in the range of 10–15 mg, were introduced and sealed in 50-μL aluminum pans (pans B014–3017 and covers B014–3003; Perkin Elmer) and stored at room temperature (22 °C). The instrument was standardized for all cooling and heating rates applied using the enthalpy and melting point of lauric acid [30]. An empty sealed pan (same as above) was used as reference. The analysis of lard was accomplished in the range of –50 °C ≤ T ≤ 70 °C. For the DSC curves, the onset and the peak maximum were determined using standard analyzing routines (see Fig. 1).

2.3 XRD as a function of temperature combined with DSC (DSC/XRDT)

XRD patterns were recorded with synchrotron radiation on the high-resolution beam line (D22) of L.U.R.E. The X-ray wavelength used was 1.549 Å (8 keV). The X-ray pattern acquisition time varied between 60 and 300 s per frame (according to the different heating and cooling rates). XRD patterns were measured in the range of –20 °C ≤ T ≤ 60 °C. The specifications and capabilities of the calorimeter used, equipped with Peltier-effect controlled sample holders, have been published [16, 17]. Briefly, two position-sensitive gas-filled linear detectors were used simultaneously and placed at a sample-to-detector distance of 0.30 m for WAXS and 1.75 m for SAXS. This setup allows the simultaneous comparison of three signals, which help to interpret the thermal events. While DSC curves provide information about the temperatures and enthalpies of thermal events, both small- and wide-angle XRDT, by the absence or presence of a set of diffraction lines, permit to identify and monitor changes of crystalline species even in very diluted systems [19]. Small-angle XRD (SAXS) peaks reflect the so-called long spacing (LS) and provide information about the longitudinal packing of the molecules [27, 28]. Wide-angle XRD (WAXS) monitors the diffraction peaks reflecting the lateral organization of the chains (short spacing, SS) [27, 28]. For TAG, the LS correspond frequently to 2, 3, sometimes 4 or 6, fatty chains, which are stacked in a parallel fashion, one on top of the other, in one crystal cell unit. LS depends on the length of the chains, their ability to pack together and the presence of “potential defects of packing”, such as unsaturations or ramifications, along the chains [18, 23, 28]. More than three crystalline subcells have been identified in the WAXS patterns of TAG, depending on the sample's thermal history and also on the unsaturated fatty chains. This fact makes interpretation difficult, especially at low temperatures, at which all TAG are nearly crystalline and because diffractions peaks are overlapping [18, 29]. First attempts to characterize thermal and structural behavior of lard using DSC, XRD and/or pulsed NMR [5–10, 16, 29] are already published. However, none of these studies provides the comprehension of both thermal and structural properties of lard. This paper represents a systematic study of the thermal and structural behavior of lard. Combined DSC and time-resolved XRDT using synchrotron radiation on the high resolution bench D22 at L.U.R.E. has been chosen for our investigation because of its resolution at small angles. The effects of cooling and heating rates on the formation of the
Fig. 1. Characteristic DSC curves representing the crystallization of lard. The influence of the cooling rate \( r_C \) is evidenced in the range of \(-0.2 \text{ K/min up to } -10 \text{ K/min, as indicated. The normalized heat flow is scaled to the cooling rate of } -10 \text{ K/min. DSC curves are shifted relatively to each other and multiplied for clarity as indicated in brackets. When not associated to } T_{\text{Onset}}, arrows indicate minor exothermic events. Thermic events are specified, when applicable, to major thermic events abbreviated as \( c_i \) (cf. Tab. 1).

Tab. 1. Crystallization and melting enthalpies of lard. The crystallization and melting enthalpies \( \Delta H \) and \( T_{\text{Onset}} \) of lard as deduced by a DSC 7 for the different thermal events. Cooling rates \( r_C \) varied from \(-0.2 \text{ K/min to } -10 \text{ K/min. Heating was performed at one heating rate } r_H = 5 \text{ K/min (according to Figs. 1 and 2).}

<table>
<thead>
<tr>
<th>( r_C ) [K/min]</th>
<th>( c_1 )</th>
<th>( c_2 )</th>
<th>( c_3 )</th>
<th>( c_1+c_2+c_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta H ) [J/g]</td>
<td>( T_{\text{Onset}} ) [°C]</td>
<td>( \Delta H ) [J/g]</td>
<td>( T_{\text{Onset}} ) [°C]</td>
<td>( \Delta H ) [J/g]</td>
</tr>
<tr>
<td>0.2</td>
<td>4.0</td>
<td>65.5</td>
<td>25.6</td>
<td>29.8</td>
</tr>
<tr>
<td>0.5</td>
<td>4.0</td>
<td>54.2</td>
<td>24.2</td>
<td>34.1</td>
</tr>
<tr>
<td>1.0</td>
<td>1.8</td>
<td>49.7</td>
<td>21.2</td>
<td>35.1</td>
</tr>
<tr>
<td>2.0</td>
<td>– 5.6</td>
<td>45.2</td>
<td>15.2</td>
<td>31.1</td>
</tr>
<tr>
<td>5.0</td>
<td>– 9.3</td>
<td>42.5</td>
<td>12.8</td>
<td>32.5</td>
</tr>
<tr>
<td>10.0</td>
<td>– 13.4</td>
<td>37.8</td>
<td>18.2</td>
<td>32.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( r_H \approx 5 \text{ K/min (after 1 min at } 50 \degree \text{C) and cooling at } r_C ) [K/min]</th>
<th>( \Delta H_{6,7} ) [J/g]</th>
<th>( T_{\text{Onset}} ) [°C]</th>
<th>( \Delta H_{3,4,5} ) [J/g]</th>
<th>( T_{\text{Onset}} ) [°C]</th>
<th>( \Delta H_{1,2} ) [J/g]</th>
<th>( T_{\text{Onset}} ) [°C]</th>
<th>( \Delta H_{1} ) [J/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>55.4</td>
<td>–10</td>
<td>24.4</td>
<td>21</td>
<td>8.2</td>
<td>36</td>
<td>88</td>
</tr>
<tr>
<td>0.5</td>
<td>50.8</td>
<td>–12</td>
<td>28.6</td>
<td>19</td>
<td>6.6</td>
<td>37</td>
<td>85.8</td>
</tr>
<tr>
<td>1.0</td>
<td>49</td>
<td>–15</td>
<td>32.6</td>
<td>18</td>
<td>5.4</td>
<td>38</td>
<td>87</td>
</tr>
<tr>
<td>2.0</td>
<td>43.4</td>
<td>–15</td>
<td>34.8</td>
<td>16</td>
<td>4</td>
<td>40</td>
<td>82.2</td>
</tr>
<tr>
<td>5.0</td>
<td>44</td>
<td>–15</td>
<td>41</td>
<td>14.5</td>
<td>0.2</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>35.6</td>
<td>–22</td>
<td>46.8</td>
<td>12</td>
<td>0.1</td>
<td>82.6</td>
<td></td>
</tr>
</tbody>
</table>

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
www.ejlst.de
1 \text{ Å}^{-1} \leq q \leq 2 \text{ Å}^{-1} \) for WAXS. This is referring to plane spacing (d) 125 Å \leq d \leq 15 \text{ Å} and 6.3 Å \leq d \leq 3.1 Å, respectively. A single computer was used for simultaneous SAXS, WAXS and DSC data collection in order to avoid temperature and/or time lags between recordings, thanks to a Visual Basic program written by P. Lesieur. X-ray calibration was obtained at 20°C with the stable β-modification of ultra-pure SSS [20] and silver behenate [31] (they perfectly agree). DSC calibration was performed as above.

### 2.4 Crystallization as a function of cooling rate

Samples were cooled from 70 to −50°C at cooling rates in the range of −0.2 K/min \geq r_C \geq −10 K/min. The crystallization was studied by DSC, and up to cooling rates of \( r_C \geq −5 \text{ K/min} \) with XRD.

### 2.5 Melting as a function of the cooling rate applied

Samples that were cooled by the above-mentioned method were kept for 1 min at \( T = −50 \text{ °C} \), then reheated at heating rates in the range of 0.2 K/min \leq r_H \leq 10 K/min. The heating was also studied by DSC/XRDT at heating rates up to \( r_H \leq 5 \text{ K/min} \).

### 2.6 Isothermal crystallization as a function of time and temperature

#### 2.6.1 DSC analysis

DSC 7 was also used to monitor the change occurring in lard samples quenched to \( −15 \text{ °C} \leq T_{\text{cond}} \leq 20 \text{ °C} \) in steps of 5 K. Typically, samples were cooled at \( r_C = −100 \text{ K/min} \) from 70°C to \( T_{\text{cond}} \) and kept for various times, ranging from 0 to 10 min, at this temperature. Then, samples were rapidly heated at \( r_H = 10 \text{ K/min} \), to minimize further changes.

#### 2.6.2 DSC/XRDT analysis

The same experimental setup was used to follow the structural changes occurring during isothermal conditionings at \( T_{\text{cond}} = −10 \text{ and 15 °C} \). Although the capillary containing the melted sample was rapidly introduced into the precooled calorimeter (quenching is about 2 s), due to the limitations of the apparatus, diffraction patterns could only be recorded after about 60 s. DSC was recorded simultaneously with XRD. Scattering was recorded for 30 min (60 s per pattern). Each isothermal conditioning was followed by a DSC/XRDT recording at \( r_H = 2 \text{ K/min} \).

### 3 Results and discussion

#### 3.1 DSC analysis

A lard sample has been systematically analyzed by DSC as a function of heating \( (r_H) \) and of cooling rate \( (r_C) \), then by DSC/XRDT using synchrotron radiation. The influence of cooling and heating rates upon the lard physical properties was first studied by DSC at all 36 combinations of the six rates used in the range of −0.2 K/min \leq r_C \leq −10 K/min. A selection of the thermal recordings obtained is presented here, together with an interpretation of the origin of the transitions thanks to the coupled time-resolved synchrotron DSC/XRDT analysis.

#### 3.1.1 Crystallization as a function of \( r_C \)

The crystallization of lard samples was studied from \( T = 70 \text{ to } −50 \text{ °C} \) as \( f(r_C) \) using DSC as described above. The recordings obtained at the six different \( r_C \) are presented (Fig. 1). The comparison of these recordings shows that (i) the DSC cooling curve of lard strongly depends on the cooling rate, (ii) at least three major peaks can be distinguished, denoted \( c_1 \) to \( c_3 \) in the order of decreasing temperature, and (iii) the onset temperature \( (T_{\text{Onset}}) \) of lard crystallization decreased from 25.6°C to 20.2°C with decreasing \( r_C \) (Tab. 1). In addition, it has been observed that the total enthalpy of crystallization decreased with decreasing \( r_C \) (Tab. 1). Peak \( c_1 \), apparently disappeared or merged with \( c_2 \) at \( r_C < −1 \text{ K/min} \). It is worth noting that this merging results from the fact that \( c_1 \) and \( c_2 \) exotherms display very different temperature dependencies. Such \( T \) dependence is even stronger for \( c_2 \), since it increased its maximum temperature from −18.6°C to 1.6°C with decreasing \( r_C \). In fact, crystallization is even more complex. At \( r_C = −1 \text{ K/min} \), up to five peaks can be distinguished (arrows in Fig. 1), showing that more than three stable crystalline organizations are obtained at lower temperatures and/or after longer times.

#### 3.1.2 Melting as a function of \( r_C \)

Samples that were crystallized at the above cooling rates were kept for 1 min at \( T = −50 \text{ °C} \), then heated at all rates indicated, corresponding to 36 recordings in the range of 0.2 K/min \leq r_H \leq 10 K/min. Only the recordings corresponding to \( r_H = 5 \text{ K/min} \) are shown in Fig. 2. As for the
influence of the cooling rate, it has been observed that (i) the transitions observed in the DSC heating curves recorded at the same \( r_H \) (here \( r_H = 5 \text{ K/min} \)) depend on the former \( r_C \) applied, (ii) the 36 DSC heating recordings obtained by varying both \( r_C \) and \( r_H \) show that they always depend on both cooling and heating rates (data not shown), and (iii) up to seven endothermic peaks named from \( h_1 \) to \( h_7 \) can be distinguished.

The first endothermic effect appeared at approximately \( T = -30 \text{ °C} \); in fact, in the range of \(-30 \) and \(-20 \text{ °C} \), depending on \( r_C \). Two main melting endotherms, the temperature positions of which are quite varying, are observed around \( 0 \) and \(-30 \text{ °C} \), respectively (Fig. 2). At \( r_C = -5 \text{ K/min} \), two overlapped endotherms \( h_1 \) and \( h_2 \), the importance of which increases with decreasing \( r_C \), are only recorded at \( T > 40 \text{ °C} \). Such endotherms, which are mainly observed at \( r_C \leq -5 \text{ K/min} \), should correspond to the formation of stable varieties. Their final melting points increase with decreasing \( r_C \), the range of which increases with decreasing \( r_C \).

3.2 X-ray analysis

Coupled time-resolved XRDT and DSC was used for the identification of the species formed during most of the \( r_C \) and \( r_H \). However, due to the apparatus limitations, lard crystallization was only investigated at \( r_C \leq 5 \text{ K/min} \), and X-ray scattering has only been measured between \( -20 \text{ °C} \leq T \leq 60 \text{ °C} \); so the full crystallization of lard could not be obtained at \( r_C > 0.3 \text{ K/min} \) (cf. Ref. [2, 8]). Only the results corresponding to boundary conditions (5 K/min and 0.3/0.15 K/min) will be presented below.

3.2.1 Fast cooling and heating

Fig. 3A and B show SAXS and WAXS X-ray pattern evolution during a fast crystallization at \( r_C = -5 \text{ K/min} \) followed by a melting at \( r_H = 5 \text{ K/min} \). The DSC recordings shown in Fig. 3C are nearly the same as obtained for the same \( r_C \) and \( r_H \) recorded with DSC 7, so we kept below the same DSC peak notations as above. The central scattering is strongly increasing while the first diffraction peak can be monitored, which is due to the occurrence of small crystals. At this rate, a first SAXS peak (48.2 Å) can be observed at \( T < 19.2 \text{ °C} (c_1) \) (Fig. 3A). A second diffraction peak (35.1 Å) appeared at \( T < 13.9 \text{ °C} (c_2) \). From \( T < -12.7 \text{ °C} \), a dominant diffraction peak appeared at 43.8 Å.
Fig. 3. Continued on next page.
Three SAXS peaks can be identified to the three main DSC peaks recorded simultaneously (denoted above as c₁ to c₃). The main diffraction lines of the various species observed are presented in Table 2.

At T ≥ 19.2 °C, only a broad WAXS peak is observed at q = 1.37 Å⁻¹, signifying that the fatty acid chains are in the liquid state. On cooling, the first WAXS peak (4.2 Å) appeared simultaneously with DSC peak c₁ at T < 19.2 °C and was attributed to the formation of some α form, thanks to the presence of a SAXS peak at 48.2 Å (Fig. 3A, B). In fact, the attribution of the crystallization to an α form rather than to a β’ form is difficult and is only deduced from the value of the long spacing observed; taking into account the fatty acid and triacylglycerol compositions, a 48.2 Å value cannot correspond in lard to a tilted-chain β’ form, but rather to an α form with vertical chains [27, 28]. The occurrence of both 4.18 and 3.83 Å peaks, indicative of a β form recorded at T < 13.9 °C, coincides with that of both the d = 35.1 Å SAXS peak and the exotherm c₂. The shift of both WAXS peaks to higher q as the temperature decreases is interpreted as a diminution of the crystalline subcell parameters. The correlation observed in their shifts shows the coupling of both peaks (see heating below). The occurrence of a SAXS peak at 35.1 Å together with that at 48.3 Å allowed the identification of the coexistence of two subcells, thanks to the high resolution of the D22 SAXS beam line. At T ≤ −12.7 °C, a series of three WAXS lines at 3.95, 4.3 and 4.4 Å, and one SAXS line at 43.8 Å corresponding to the crystallization peak c₃, indicate the formation of a new species. It is worth noting that, again, SAXS lines appeared subsequent to the DSC peak and the WAXS lines were observed soon after. This can be interpreted as a delay in the TAG layer organization after self-organization of the chains. In other words, methylene groups of the chains organize first, before the whole TAG molecules. However, as the sample was kept at −20 °C for several minutes, we observed that the WAXS lines substantially developed at this temperature, showing that at this cooling rate crystallization is incomplete (cf. Ref. [2, 8]). This is in line with the observation that at r_c = −5 K/min, all WAXS peaks are observed on the top of a broad scattering “line” centered at q = 1.37 Å⁻¹ (d = 4.6 Å), indicative for the presence of a rather high amount of liquid phase or disorganized structures. The WAXS pattern of the pure β form of tristearin is reported in Figs. 3B and 4B for comparison to that of lard. The presence of this “liquid” or “disordered” phase at −20 °C, probably related to the broad TAG composition (differences in chain length and unsaturation likely lead to a loose packing), explains the polymorphic changes observed above.
Upon heating, in the range of −20 to 15 °C, the diffraction line at 43.8 Å, apparently coupled with those at WAXS 4.3, 4.4 and 3.95 Å, partly vanishes as shown in Fig. 3A and C during the corresponding h6 and h7 endotherms. Contrary to the diffraction line at 43.8 Å, the WAXS lines completely disappeared. The h6 endotherm was related to the decreases of lines at 3.8 Å correlated with 48.2 Å in the range of 15 °C ≤ T ≤ 25 °C. The vanishing of the diffraction line at 35.1 Å coupled with that at 4.15 Å leads to the h2 and h3 endotherms, although this does not explain their occurrence. During the set of h1 and h2 endotherms (distinct in Fig. 5), only one SAXS diffraction line can be distinguished at r C = 0.5 K/min (data not shown). The transitions occurring during isothermal conditioning of lard were also monitored using both DSC and DSC/XRDT using synchrotron radiation.

### 3.2.2 Slow cooling and heating

An example for the evolution of X-ray scattering at r c = −0.15 K/min followed by a heating at r h = 0.3 K/min is given in Fig. 4A and B. A first weak scattering peak (45.4 Å) can be distinguished at about 26 °C (data not shown). At 24 °C, this peak rapidly vanishes and splits into two weak peaks of longer (49.6 Å) and shorter distances (44.2 Å), respectively. Both peaks vanished within the 2 K scan, while a very broad and important scattering is observed at about q = 0.05 Å⁻¹. This effect can also be distinguished at r c = 0.5 K/min (data not shown). The peak at the shorter distance progressively shifts to 43.8 Å. A second-order peak appeared at d = 34.7 Å from T = 22.7 °C, shifting to 34.3 Å at lower T. The intensity of the scattering at 43.8 Å rose strongly at T < 6.5 °C, while a second-order peak is observed at 21.9 Å. It is worth noting that the half width heights of both SAXS peaks at 43.8 and 34.5 Å are very different; while the line at 43.8 Å is very sharp, the half width of the peak centered on 34.5 Å is about four times broader, indicating a highly disordered structure for the latter.

At wide angles, the scatterings of two apparently coupled peaks appear at 4.15 and 3.85 Å from T < 22.7 °C (c3), indicating the formation of a β’ chain packing (Fig. 4C). Then, a line at 4.61 Å rose at 20.7 °C, showing the occurrence of some β packing. Another three peaks (4.4 and 4.3 and 3.95 Å) appeared separately at negative temperature. All six WAXS lines got very sharp, and their position and intensity was monitored vs. T. While the characteristic β’ lines (3.8 and 4.14 Å) exhibit a strong positive linear T dependence of about 2% within 50 °C, the β line does not change its position. The dependence of the other diffraction peaks is more complex, since the line at 3.95 Å is reaching a maximum around 0 °C while the one at about 4.3 Å exhibits the reverse behavior and the 4.4 Å line is almost not changing. The behaviors of all six lines are reversible with T. The β lines, observed at 4.6 Å and around 3.9 Å, persist at T ≥ 37.8 °C, showing that the phase likely formed by trisaturated TAG is the most stable.

All data show that the crystalline structure strongly depends on the sample time and the temperature history. Several polymorphic forms of β and β’ type, and possibly α type, are observed. The occurrence of these crystalline varieties depends on the cooling rate, showing that lard exhibits a strong polymorphism. The crystallization extending at T < −20 °C and the enthalpies of the DSC peaks recorded demonstrate the existence of an important liquid phase together with the crystalline phases described above at least down to −20 °C (Fig. 1). While this liquid phase rapidly contributes to reaching the formation of stable varieties and thermodynamic equilibrium (Ostwald ripening), polymorphic transitions can be monitored even at fast scanning rates (about 5 K/min) using the brightness of synchrotron radiation.

To further evidence the polymorph changes that may occur during thermal treatment of lard, complementary isothermal conditionings were performed and recorded.

### 3.3 Isothermal conditionings

The transitions occurring during isothermal conditioning following rapid crystallization of lard were also monitored using both DSC and DSC/XRDT using synchrotron radia-
A

SAXS

V-shaped

001

49.0°C
38.8°C
28.6°C
18.4°C
8.3°C

002

Heating
at \( r_c = 0.3 \text{K/min} \)
-1.9°C
-22.3°C

Crystallization
at \( r_c = 0.15 \text{K/min} \)
-12.8°C
-2.6°C

7.5°C
17.6°C

B

WAXS

\( \beta = 4.6 \text{Å} \)

\( \beta_1 \)

\( \beta_2 \)

1.10
1.20
1.30
1.40
1.50
1.60
1.70
1.80

\( q [\text{Å}^{-1}] \)
3.3.1 DSC monitoring

The DSC recordings obtained after conditioning times ranging from $t_{cond} = 0$–10 min are shown in Fig. 5A, B. After $t_{cond} = 0$ min and isothermal conditioning at $T_{cond} = 15^\circ C$, almost no crystallization of stable species melting at $T \geq 30^\circ C$ could take place, as shown by one single endotherm recorded at about $25^\circ C$ (Fig. 5A). Neither stable forms, such as $h_1$ or $h_2$, nor $h_3$ is formed. On the contrary, after 1 min of conditioning, $h_3$ and $h_2$ or $h_1$ endotherms are recorded in addition to the melting of unstable forms. Longer conditioning (2 and 10 min) leads to increasing $h_1 + h_2$ peaks. The same behavior is observed upon heating at $r_H = 10$ K/min after $T_{cond} = -10^\circ C$ conditioning (Fig. 5B). A set of melting endotherms was always recorded, and the formation of some stable form was observed whatever the conditioning time was. Comparison of Fig 5A and B shows that with $T_{cond} = 0$ min, stable forms only crystallize at temperatures below $15^\circ C$.

The endotherms $h_1$ and $h_2$, corresponding to the melting of stable varieties, increase in enthalpy as the conditioning time at $15^\circ C$ increases (Fig. 5A). Taking into account the fast scanning rates, especially upon heating, which further limit the evolution of the crystals, the comparison of the enthalpies of these peaks allows the determination of the crystallization rate of these varieties at $15^\circ C$, which is $\frac{dH(h_1,2)}{dt} = 0.66 \pm 0.02$ mJ g$^{-1}$ min$^{-1}$.

Surprisingly, the amount of crystal formed shown by the enthalpies of the $h_3$ endotherm decreases with increasing conditioning time within 5 min at $-10^\circ C$, while the enthalpies of the $h_1$ and $h_2$ endotherms do not change considerably after $t_{cond} = 1$ min.

3.3.2 DSC/XRDT

Similar experiments were performed using DSC combined with synchrotron radiation XRD, as described in the Materials and methods section (only the isothermal conditioning at $-15^\circ C$ is shown). Fig. 6 nicely illustrates the monitoring of the phase transition $x$ to $y'$ at $T = -15^\circ C$. A remarkable aspect of this phase transition is the persistence of the main line of the WAXS pattern at about 4.15 Å, which corresponds to both hexagonal and
orthorhombic subcells, while that of the SAXS pattern at 48.2 Å vanishes at least for 1 min before that of 43.8 Å appears. This makes us conclude that during the transition corresponding to the change in chain tilt, the lateral order in the chain packing is at least partially maintained while the stacking order between lamellae is lost. The passage through an intermediate liquid crystalline-like state cannot be ruled out. During this transition, an exothermal process is recorded by DSC, the onset of which is at 8 min and its maximum is at about 11 min from the beginning of the isothermal experiment. This phase transformation process is extremely rapid and energetically “concentrated”, there-
fore easily detected (ΔH = 6 mJ). This value should be taken with caution since the recrystallization process is very slow and not yet completed after 25 min, as shown in Fig. 6. This experiment further evidences that such a phase transition may occur at low temperatures and not necessarily upon heating, thanks to the presence of the liquid phase discussed above. Comparing the surface of the diffraction peaks at both SAXS and WAXS, which are related to the amount of crystalline mass, before and after the transition, it can be observed that about twice more crystals are formed 10 min after the transition than at 5 min before the onset of the DSC exotherm. Then, the observed endotherm corresponds in fact to both the transition and a distinct enthalpy of crystallization.

3.4 Packing and electronic density

The SAXS diffraction lines (001, 002) attributed to the fraction with the highest melting point (h₁₂) are observed at d = 43.8 Å (see Figs. 3, 4). This let us conclude that this 2L structure is formed by trisaturated triacylglycerols representing less than 10% overall, such as SPS and its isomer PSS. The thickness of the β-bilayers expected for SPS and PSS is 43.4 Å and 46.5 Å, respectively [27]. This and the persistence of the line at 4.6 Å on the recording at the slow heating rate (Fig. 4B) make us conclude that the structure associated with the fraction with the highest melting point derives from SPS and is of β 2L type.

The interpretation of the DSC and XRD data associated with the endotherms h₃₄₅ and h₆₇ is more difficult. Two β₁' forms with different melting points [Tm(β₁')] < Tm(β₁) are observed in WAXS in this melting domain. In fact, in SAXS recordings, three lines are coupled with these two β₁' forms. Fig. 3C shows that (i) the β₁' form is associated with the line at 43.8 Å and endotherm h₁, and (ii) the lines at 35.1 and 48.2 Å contribute to the β₁' form and endotherms h₃₄₅. Regarding the attribution of h₃₄₅ and h₆₇, which melt in the range of 20–30 °C and 0–10 °C, respectively, to a triacylglycerol group, it is expected that those correspond to monounsaturated and/or di-unsaturated triacylglycerols, respectively.

The fact that the diffraction peaks of this lowest and highest melting fraction only show distances of about 35 Å is puzzling. This value is too low to correspond to a 2L packing, unless the chain tilt is very important. Taking into account the fatty acid and triacylglycerol composition of lard, we conclude that the observed diffraction at 35 Å does neither correspond to a first order of a 2L packing nor to that of a 3L packing. In spite of a lack of scattering/diffraction in the region of 0.085 Å⁻¹, the attribution of the diffraction line observed at 35 Å to the second order of a 3L packing of 70 Å cannot be ruled out (Fig. 4A). A quasi-perfect symmetry of the stacking layer might explain the absence of first order.

In fact, depending on the thermal treatment, the scattering in the domain 0.05 Å⁻¹ ≤ q ≤ 0.013 Å⁻¹ is either U- or V-shaped (arrows in Figs. 3A and 4A). The V-shape present at q centered on 0.08–0.09 Å could also be due to the presence of the first- and second-order diffraction peaks at q = 0.06 and 0.12 Å⁻¹ of the lamellar structure observed at 35 Å (q = 0.18 Å⁻¹), since they both appear and disappear simultaneously with this peak (in this hypothesis, the stacking period should be 105 Å) (Fig. 4A).

An alternative explanation would be that the bump around q = 0.06 Å is a scattering peak, related to crystal sizes, which partly overlap first order of the line at about 35 Å. The U-shape present in the same q range is observed at fast scanning rates and would reflect a less organized structure coming along with broader and weaker diffraction lines.

4 Conclusion

As a summary, we are not able to explain the molecular organization that is reflected by the diffraction peak at 35 Å. Such an organization is made by the monounsaturated TAG (sat., sat., O and sat., O, sat.) that represent about 30% of the fat. Whichever the stacking period, the partial extinction of the first- (and possibly second-) order peaks of a diffraction line at 70 or 105 Å (35 Å) could be related to the specific distribution of the unsaturated fatty acids of lard on the glycerol. Since the diffraction line at 35 Å is not observed for other fats with corresponding fatty acid composition like palm (note that, except for the palmitic/stearic fatty acid ratio, these two fats have similar compositions), one can imagine that a “phase separation” between layers of sat., sat., O and sat., O, sat. triacylglycerols would lead to an alternate structure due to oleic acid esterified in the 2- and 3-position, which might explain the particular electronic density profile.

The fact that structural transitions take place after rapid crystallizations even at low temperatures was related univocally to α to β₁' prime transition. Taking into account the large difference in molecular volume of the two subcells to which these varieties correspond, the industrial consequence of this polymorphic evolution is the predictable contraction of the fat after fast cooling. Another practical consequence reflected by this work is that lard fractionation would be rather difficult, because the temperature lag between c₁ and c₂ crystallizations only occurs at high cooling rates, resulting in small crystals, while two crystalline varieties are formed simultaneously upon slow cooling in c₂.

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim www.ejlst.de Systematic investigation of lard polymorphism 605
Then, we conclude that the coupling of DSC and XRDT allows at least the partial identification of the structures developed during the thermal treatments, whatever the rate, fast or slow, which permits the attribution of the thermal events recorded by DSC. Once this identification has been established, conventional analysis can be undertaken simply using commercially available DSC.

Acknowledgments

We thank L.B.C. for providing lard samples and J.-L. Vandevouër (CTSCCV, Maisons-Alfort, France) for interest in this work. We thank ACTIA for funding this research and the initial work of D.K. We also thank our group members for their always very fruitful discussions.

References

[1] L. DeMan, V. D’Souza, J. M. DeMan, B. Blackman: Poly- 
morphic stability of some shortenings as influenced by the 
fatty acid and glyceride composition of the solid phase. J 
2733149, 1956.
[4] E. S. Lutton, M. F. Mallery, J. Burgers: Interesterification of 
mechanics of sodium methoxide treatment of lard. J Am Oil 
chemical interesterification on the physicochemical proper-
ties of complex fat systems. 2. Morphology and poly-
[7] K. A. Al Rashood, R. R. A. Abou-Shabaan, E. M. Abdel-
Moety, A. Rauf: Compositionnal and thermal characterization of 
genuine and randomized lard: A comparative study. J Am Oil 
fractions at 15 °C. Relationship with thermal behavior and 
[9] D. Chapman, A. Crossley, A. C. Davies: The structure of the 
major component glyceride of cacao butter, and of the major 
Differential scanning calorimetric analysis for determination of 
some animal fats as adulterants in palm olein. J Food 
Lipids 2003, 10, 63–79.
[11] O. T. Quimby, R. L. Wille, E. S. Lutton: The glyceride com-
of the glycerides and the nature of the fatty acids in the 
feeds on the composition of the depot fat of pigs. Biophys J. 
Karleskind, J. P. Wolff, Lavoisier ed. Technique et Doc- 
rate on the structure and mechanical properties of milk fat and 
Investigation of the complex thermal behavior of fats. J 
Loisel, M. Ollivon, C. Bourgaux, P. Lesieur: DSC and X-ray 
[18] F. Lavigne, M. Ollivon: Differential scanning calorimetry and 
dynamic X-ray diffraction studies of polymorphic transitions 
fat crystallization in aerated food emulsions by combined 
DSC and time-resolved synchrotron X-ray diffraction. Food 
[21] M. Ollivon, R. Perron: Etude de melanges binaire de trigly-
erides derives des acides palmitique et stearique. Chem 
[22] M. Ollivon, R. Perron: Measurements of enthalpies and 
entropies of unstable crystalline forms of saturated even 
[23] Y. Gibon, F. Durant, C. Deroanne: Polyphosphoryl and inter-
solubility of some palmitic, stearic and oleic triglycerides – 
transitions and polymorphism of cocoa butter. J Am Oil 
Chemistry of Lipids. From Alkanes to Phospholipids. Plenum 
In: Manuel des Corps Gras. Eds. A. Karleskind, J. P. Wolff, 
Lavoisier ed. Technique et Documentation, Paris (France) 
comparison of sodium methoxide-treated lards. J Am Oil 
phospholipid-water systems. J Colloid Interf Sci. 1983, 95, 
471–482.
diffraction analysis of silver benenate, a possible low-angle 

[Received: May 12, 2005; accepted: August 9, 2005]