Crystal Morphology of Mixtures of Tripalmitin and Butterfat

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ABSTRACT: Blends of butterfat and tripalmitin are of interest for use as moisture barriers in edible films. Tripalmitin was blended with butterfat in the ratios 100:0, 90:10, 80:20, and so on through to 10:90 and 0:100. Crystal morphologies of squash and smear preparations were determined by polarizing light microscopy. Samples were stored at 19–22°C and re-examined after 6 d and after 30 d. Morphology was strongly dependent on composition and the presence of a coverslip. Morphology was less dependent on polymorphic form and age. Barrier properties depend more strongly on morphology than on polymorphic form.

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KEY WORDS: Edible films, mixed crystals, polarizing light micro-scopy, thin films, triglycerides.

Mixtures of tripalmitin and butterfat can be used as alternatives to hard butterfat fractions in many applications (1,2). In this study, we are interested in using these mixtures in edible films based on protein or polysaccharide, to enhance the relatively poor moisture barrier properties of these biopolymers. There are two principal types of composite film—emulsion and bilayer. In the former, the fat is dispersed as an emulsion and, in the latter, a layer of fat is sandwiched together with a layer of biopolymer. Bilayers provide the best barrier to the transport of moisture (3,4). The ratio of solid fat to liquid fat, as well as the size, shape, and orientation of the crystals in the fat layer, will affect the ease with which molecules such as water and oxygen pass through the barrier (5–7). The purpose of the work reported here was to determine the crystal morphology of thin layers of mixtures of tripalmitin and butterfat, and to relate the morphology to the barrier properties. This characterization also will be useful in any application where blends of tripalmitin and butterfat might be used as alternatives to hard butterfat fractions.

EXPERIMENTAL PROCEDURES

Preparation of blends. Blends were prepared from anhydrous butterfat that was a gift from Mid-America Dairymen, Inc. (Springfield, MO). Tripalmitin (stated purity 95%) was obtained from Fisher Scientific (Pittsburgh, PA), and was used without any further purification. Gas-chromatographic analysis indicated a purity of 94%, and the principal impurity was identified as trilaurin. Tripalmitin and butterfat were mixed in the ratios 100:0, 90:10, 80:20, and so on through to 10:90 and 0:100. The blends are referred to in the text by the ratio of tripalmitin to butterfat. Thus, "the 20:80 blend" indicates butterfat containing 20% added tripalmitin by weight. Blends were heated to not less than 90°C for 30 min before use in order to destroy any existing crystal nuclei.

Preparation of thin films from blends of tripalmitin and butterfat. The triglyceride layer in edible bilayer films is typically 0.1–0.2 mm thick (8), which is too thick to properly examine under the light microscope. Thin layers of fat were therefore prepared especially for the light microscope, by a squash method and a smear method. The squash method was chosen because squashes have been widely used for fats, and thus the results could be compared with published data. The smear method was chosen because it is analogous to the technique used to produce edible bilayer films. Bilayers are prepared by placing a small volume of molten lipid on top of a previously formed biopolymer film. A heated rod (such as a curling iron) is used to draw the lipid out into a film of controlled thickness, which cools rapidly (for more details see Ref. 1).

A glass slide was heated to 50–60°C and a hot glass pipette was used to transfer a small volume (about 25 μL) of triglyceride to the slide in each preparative method. Conventional squash samples were prepared by placing a coverslip over the lipid and allowing it to settle under the influence of its own weight and the surface tension of the molten fat. Smear samples were produced by drawing the lipid along the length of the slide by using a second slide held at a 45° angle to the first. Both smears and squashes were placed on a metal plate for rapid cooling. Samples were examined between crossed polarizing filters under a Zeiss Axioplan Universal microscope with MC-100 camera (Carl Zeiss, Oberkochen, Germany). Samples were stored at 19–22°C and re-examined after 6 d and again after 30 d. Duplicate squashes and smears were examined for each blend. Unless otherwise noted, the photomicrographs show a typical field for each sample.

RESULTS AND DISCUSSION

Freshly prepared samples. Tripalmitin squash samples had a strongly birefringent pattern (Fig. 1a). It is not possible to

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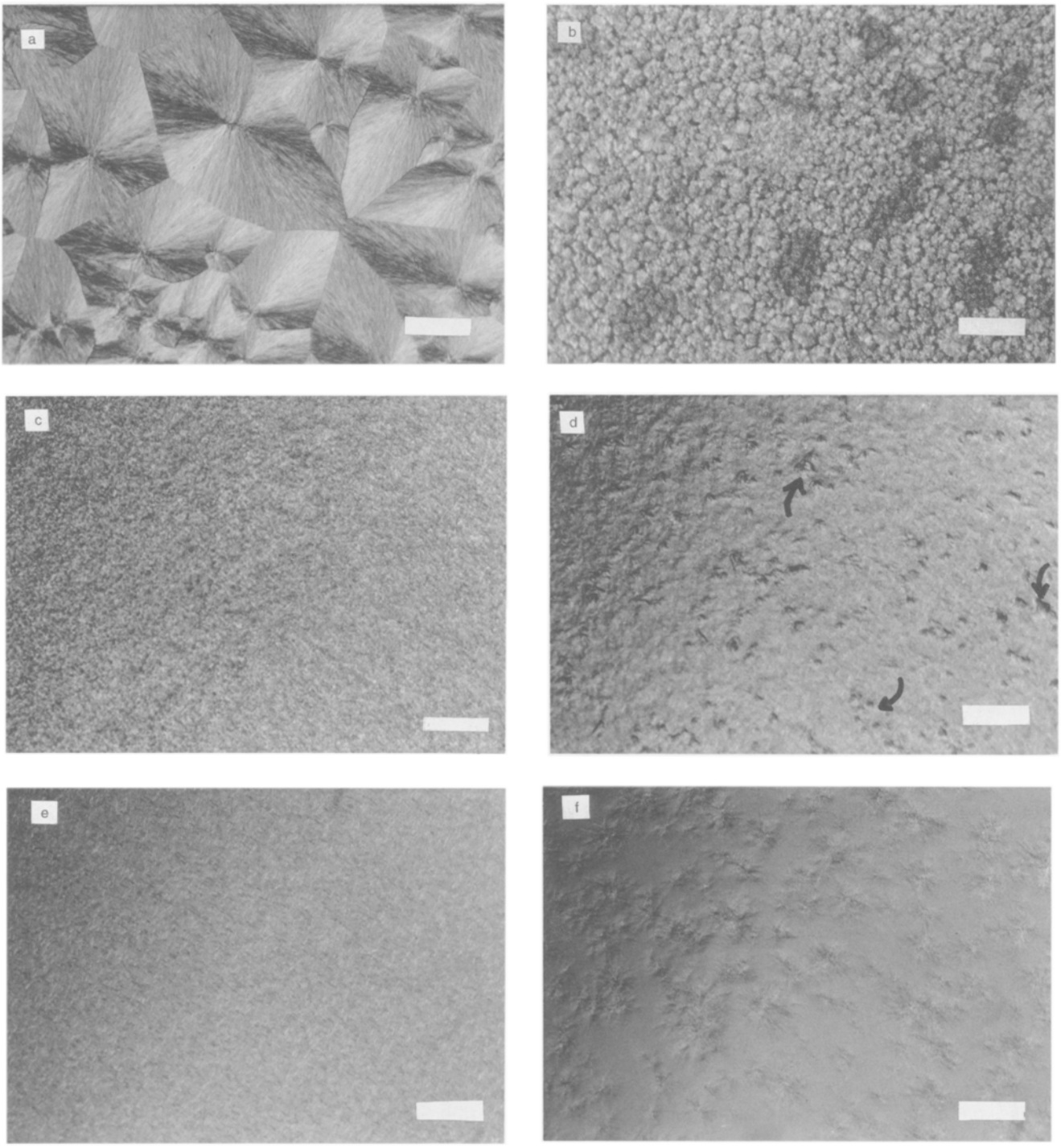

FIG. 1. Polarizing-light micrographs of fresh samples. Each bar represents 50 μm; a, tripalmitin squash; b, tripalmitin smear; c, tripalmitin-rich (70:30) smear; d, butterfat-rich (10:90) smear (arrows indicate mixed crystals of tripalmitin and high-melting component of butterfat); e, butterfat-rich (20:80) squash; and f, butterfat smear.

identify the polymorphic form of tripalmitin unambiguously from the crystal morphology (9). The pattern seen here could indicate either the β′ polymorphic form (Fig. 1d and 1e of Ref. 9) or the α form (Fig. 2a of Ref. 9). Differential scanning calorimetry of films similar to the ones used here indicates that tripalmitin crystallizes initially into the α form, but is fully converted to the β form after one week (1). The polymorphic form of the actual samples examined here was not determined, so the discussion will therefore be confined principally to morphology.

The birefringence was less marked in the smear sample (Fig. 1b), and the spherulites were much smaller. The presence of a coverslip clearly had a strong influence on crystal nucleation and growth rate.

Blends rich in tripalmitin (90:10, 80:20, 70:30, 60:40) had a fine-grained appearance in smear preparations (Fig. 1c). Squash samples (not shown), however, appeared much more like the tripalmitin squash samples of Figure 1a, especially in areas near the edge of the coverslip.

Blends that were rich in butterfat (50:50, 40:60, 30:70, 20:80, 10:90) showed a clear separation of crystalline material in smear samples (Fig. 1d). The crystals were small, coarse, and irregularly shaped. They were probably composed of tripalmitin and the high-melting component of the butterfat. These crystals were not noted in squash samples, where the coverslip promoted a more organized crystal network (Fig. 1e).

Squash samples of pure butterfat (not shown) had no visible structure under the microscope, but smear samples (Fig. 1f) contained a spidery network, which is typical of crystalline butterfat (10,11). Liquid fat was evident as featureless areas on the photomicrograph. In every blend, from pure tripalmitin to pure butterfat, the presence of a coverslip had a dramatic effect on the crystal morphology.

Six-day-old samples. Tripalmitin samples were largely unchanged in appearance in both the smear and the squash preparations. The graininess of the tripalmitin-rich smear samples became more marked upon storage, as the crystallites became more clearly defined. The degree of organization in the squash samples became much less marked, so that most areas of the slide looked like the smear sample, although some areas retained their original appearance.

The separation of crystalline material from the butterfat matrix became more noticeable in the mixtures rich in butterfat, especially in the 10:90 smear sample (Fig. 2a). This suggested that the crystals acted as nuclei, allowing more of the high-melting component of the butterfat to crystallize out. This separation was not evident in the squash samples, which did not change much.

The crystal network became more fully developed in the butterfat smear sample, and a finely grained appearance was observed in some areas of the butterfat squash sample (Fig. 2b).

Thirty-day-old samples. The appearance of the tripalmitin squash samples was still largely unchanged after 30 d (Fig. 3a). Tiny, needle-shaped crystals were observed in many areas of the smear samples (Fig. 3b), which suggested that there was a slow change in crystal morphology in these samples.

Tripalmitin-rich smear samples also developed a more needle-like appearance, but the needles were not as well-defined (Fig. 3c). This structure was also apparent in most of the squash samples, except in areas near the edge of the coverslip. In the butterfat-rich samples, the backgrounds of the smear samples became more organized, which was probably associated with the very slow crystallization of some components of the butterfat. The 10:90 smear sample showed a pronounced separation of crystalline material from the background (Fig. 3d). The large amount of crystalline material in the photomicrograph suggested that the crystals were composed largely of high-melting fraction from the butterfat. Squash samples of the butterfat-rich blends mostly appeared similar to the smear samples, although some areas were still grainy.

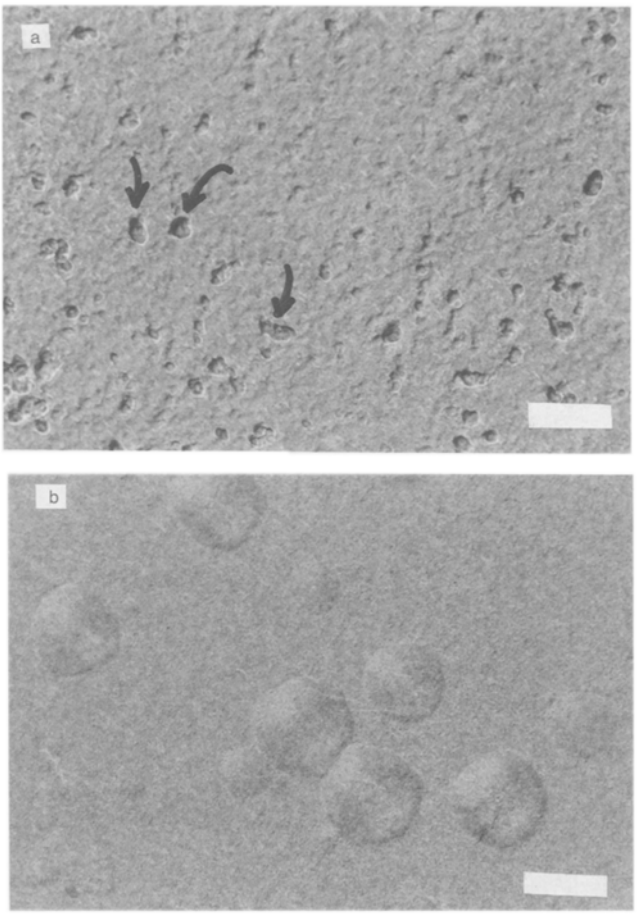

FIG. 2. Polarizing-light micrographs of six-day-old samples. Each bar represents 50 μm; a, butterfat-rich (10:90) smear, mixed crystals (indicated by arrows) are prominent; and b, butterfat squash.

Smear preparations of butterfat (Fig. 3e) showed the maturation of the crystalline network seen in Figure 1f. The crystallization process for butterfat clearly took a long time to complete at room temperature. The squash samples remained largely unchanged.

Crystal morphology and barrier properties of edible films. Crystal morphology depended primarily on composition and on the presence of a coverslip, and less on the age of the sample. It is likely that the morphology of crystals in edible bilayer films is more like that of the crystals in the smear preparations examined here than in the squash samples, because of the way in which edible bilayer films are prepared. The differences between squash samples and smear samples indicate that much of the published microscopic data are of limited

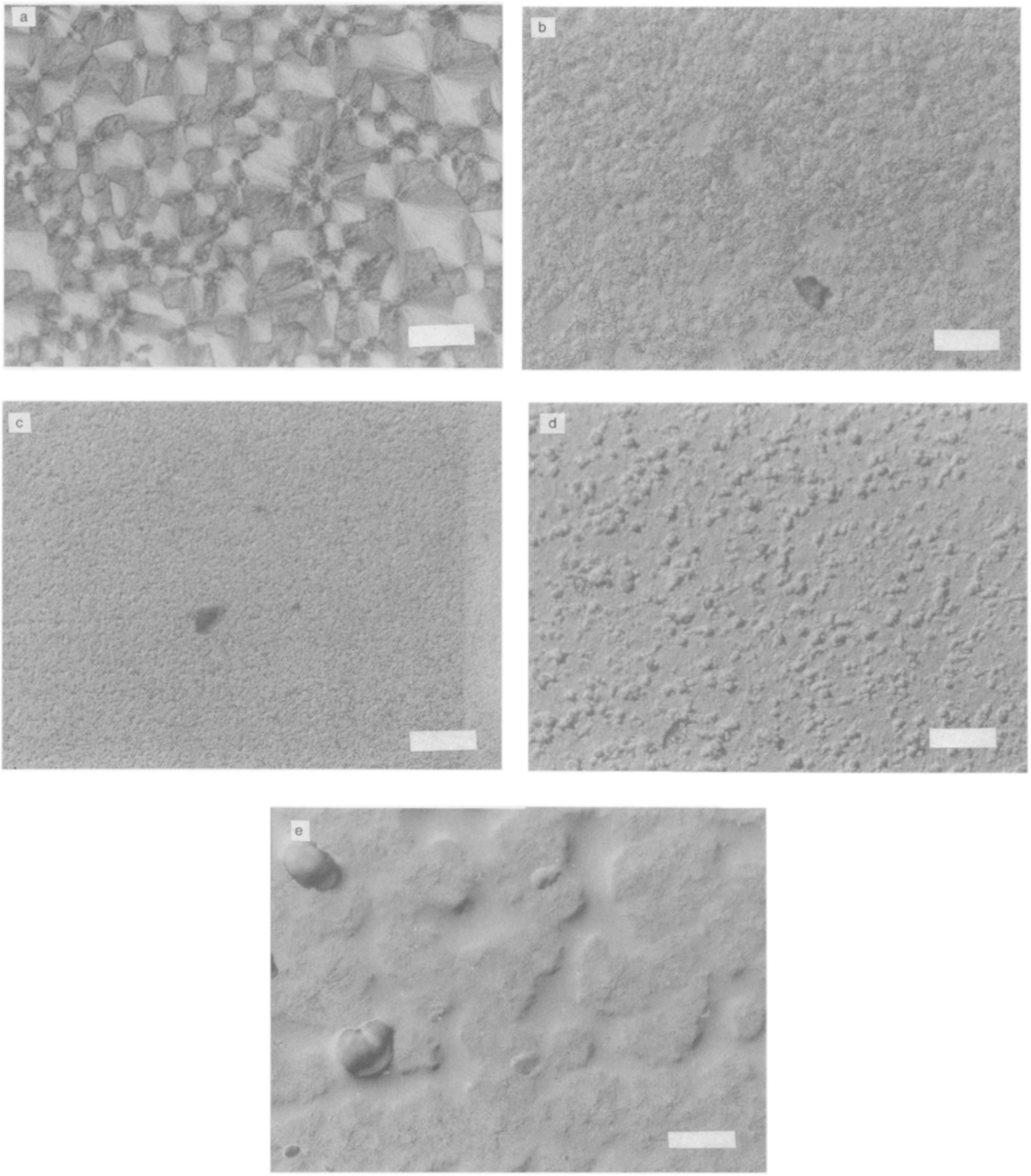

FIG. 3. Polarizing-light micrographs of 30-day-old samples. Each bar represents 50 μm; a, tripalmitin squash; b, tripalmitin smear; c, tripalmitin-rich (70:30) smear; d, butterfat-rich (10:90) smear; and e, butterfat smear.

relevance to edible films. It is important to choose a preparative technique that correlates closely with the way the fat is treated in any given application.

There is a strong correlation between the water vapor permeability (WVP) and the composition of edible films formed from blends of butterfat and tripalmitin (8). The WVP decreases exponentially with the mass fraction of tripalmitin in the film (x) according to:

$$\text{WVP (g mm/kPa h m}^2) = 0.3653e^{-3.629x} \quad [1]$$

with an r^2 of 0.9467 (Shellhammer, T.H., and J.M. Krochta, unpublished manuscript). As the morphology changes from the isolated crystals (seen in butterfat-rich samples) to the crystal network (seen in tripalmitin-rich samples), the permeability drops considerably. Once a continuous, dense crystalline network is formed, addition of further tripalmitin makes a relatively small difference to WVP. Although only small amounts (10–20%) of tripalmitin need be added to simulate fractionation of butterfat (1), relatively large amounts (60–70%) need to be added to achieve the dense crystalline network required for good barrier properties.

The development over time of the network (Fig. 3) suggests that the barrier properties of films based on mixtures of tripalmitin and butterfat might improve slightly with time. The strong influence of the coverslip on the morphology suggests that "sandwich" films (where a layer of fat is sandwiched between two layers of biopolymer film) might have different barrier properties to bilayer films.

Influence of polymorphic form on barrier properties. Kester and Fennema (12) investigated thin layers of hydrogenated vegetable fat, which were cast on filter paper supports. They tempered the fat from the α form into the β′ form and then into the β form, to determine the influence of polymorphic form on the barrier properties. It would be expected that barrier properties would increase in the order $\alpha < \beta' < \beta$, because the density of the fat increases as the polymorphic form changes. However, the oxygen barrier properties increased in the order $\beta' < \alpha < \beta$, and the water barrier properties increased in the order $\beta' < \beta = \alpha$. These results could not be fully explained at the time (12).

Kellens *et al.* (9) observed that a tripalmitin sample that had been rapidly cooled into the α form retained the same microscopic appearance upon being slowly tempered into the β form. We have seen that morphology influences barrier properties. An increase in density (due to a change in polymorphic form) with no apparent change in morphology could imply the shrinkage of some crystallites and the creation of tiny voids and cracks that could compromise the barrier properties. Thus, it would seem that the results of Kester and Fennema (12) may be explained (at least in part) by the influence of crystal morphology on the barrier properties, which seems to outweigh that of the polymorphic form. It is clear from this study and the studies of Kellens *et al.* (9), Kester and Fennema (12), and Shellhammer and Krochta (unpublished manuscript) that the morphology of the crystals has a much larger influence on barrier properties than does the polymorphic form.

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