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The effects of phospholipids on crystallisation and crystal habit in triglycerides

Many food products contain a network of fat crystals. The sizes and shapes of these triglyceride crystals are extremely important in producing the good physical properties and texture for a high-quality final product. Control of crystal habit can affect a fat's structure and hence the resultant behaviour. In this work the effects of phospholipids on the crystal habit of triglyceride crystals have been investigated. Optical and scanning electron microscopy, DSC, and X-ray diffraction have been used. The effects of certain phospholipids on the crystallisation of fats have been shown, and large changes to crystal habit have been demonstrated. These changes are caused by the interactions of the phospholipid molecules with the crystallising triglyceride molecules. Interference with the crystallising molecules causes changes to the shape and size (habit) of the resultant fat crystals. Effects on nucleation and crystal growth are demonstrated. These dramatic changes to the crystal habit can have a significant effect on the properties of the resultant fat system. For example the process of fat fractionation could be significantly enhanced. Alternatively, the network structure of fatty products could be controlled or altered opening up the possibility of the development of new and improved products.

Keywords: Triglyceride, habit, crystallisation, phospholipid.

Research Paper

1 Introduction

Triglyceride crystallisation is a complex phenomenon that has interested researchers for many years. It is complicated by the slow growth rates and the polymorphic behaviour of fats. Despite this the behaviour is fairly well-known and a useful review is that edited by *Garti* and *Sato* [1].

The effects of impurities and emulsifiers or surfactants further complicate triglyceride crystallisation, whether they are present by accident or design in a system. They may be added to a system for emulsification or surfactant behaviour or they may be present as natural impurities. Some work has been published on the effects of different emulsifiers on fat blends [2–4]. However, there appears to have been little systematic work to understand underlying interaction mechanisms. *Wähnelt* et al. [5, 6] have shown that the addition of diglycerides can retard the crystal growth of cocoa butter. The author has investigated the effects of partial glycerides on model fat systems [7, 8]. In these papers models for the interactions of impurities and additives with crystallising fats have begun to be developed. It has been shown that the shape and size of the additive molecule are critically important in determining its interaction with the crystallising triglyceride molecules. The nature of the effect that occurs is dependent upon the relative sizes and shapes of the different molecules. Effects on both the growth rate and resultant crystal shape occur.

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The polymorphic form, shape, and size of the fat crystals are important in ensuring that a fatty product has the required physical properties. Great care is often taken during product processing in order to achieve optimal product properties.

The general crystal morphologies of the three major polymorphic forms of triglycerides have been well characterised for a great number of years. In 1960 *Hoerr* [9] described the three forms thus:

α: Very thin, less than several μm in size, smooth surface.
β': Long needle shape (Size distribution < 5 μm).
β: Large, plate-like crystals, 20–100 μm in size.

More-detailed morphologies of β crystals of different triglycerides have been determined by various authors [10–11].

Thus, the change from one polymorphic form to another leads to a very large modification to the crystals' morphology. However, by controlling crystallisation of a polymorphic form, more subtle changes of habit can be achieved. Molecules that affect the crystallisation are likely to interact in different ways with the different faces of a growing crystal, so that crystal habit modification can be achieved. This process is well established in the chemical industry

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as a means for modifying crystal habits, as discussed by *Lewtas* et al. [12]. However, there has been very little systematic attempt to couple measurements on the effects of additives on the crystallisation of triglycerides with any effects on habit modification.

Phospholipids are very commonly used as emulsifiers and surfactants in fat-based systems. Interactions between phospholipids and triglycerides are therefore important. Effects of phospholipid molecules on triglyceride crystallisation and crystal habit could be particularly important in many systems.

The objective of this work is to consider the effects of phospholipids on the crystallisation behaviour of both model and natural fats. The effects of the interactions between the molecules have been studied and effects on crystallisation and crystal habit have been noted. Thus, interaction models can be developed and so potential applications for the use of phospholipids in foods processing determined.

2 Materials and methods

Phospholipids (soy bean lecithin and pure phosphatidyl choline [1-2-diacyl-*sn*-glycero-3-phosphocholine], phosphatidyl ethanolamine [1,2-diacyl-*sn*-glycero-3-phosphoethanolamine] and phosphatidyl inositol [1,2-diacyl-*sn*-glycero-3-phospho-[1-D-myo-inositol]], as well as phosphatidyl cholines of differing chain length) and pure glycerides were obtained from *Sigma Chemical* Co., Stockholm, Sweden. The phospholipids used were from natural sources of soy bean, bovine brain, and egg yolk. Additionally, synthetic phosphatidyl cholines with controlled side chain composition were obtained. Materials with side chains of C_{12} to C_{18} were obtained and used. *Karlshamns AB,* Karlshamn, Sweden supplied palm oil.

Mixtures of 0.1, 0.2, 0.5, and 1 weight-% of phospholipids were prepared in palm oil, trilaurin, and tristearin. Samples were heated to approximately 80 °C, with stirring, and dissolution of the additive was ensured.

2.1 Initial screening

Mixtures of 20 ml of palm oil with and without phospholipids were heated to 80 °C and stirred until dissolution of the phospholipid had occurred. They were then left overnight at 20 °C. The mixtures were then observed and samples taken for microscopy. A control sample containing no phospholipid was also prepared and tested in the same manner.

2.2 Temperature gradient microscopy

A temperature gradient microscopy system was designed and built according to the manner of *Hunt* et al. [13]. The system was used as previously described [7, 8]. Samples of trilaurin plus 0.2 weight-% phosphatidyl ethanolamine and phosphatidyl choline were studied. Samples were studied at forced crystallisation rates of between 10 and 100 °C per hour.

2.3 Scanning electron microscopy

A portion of the material from the initial screening was taken and as much liquid as possible was filtered off. The sample was then thoroughly washed with acetone at the same temperature to remove all remaining traces of oil without damaging the solid. The remaining solid was then quenched in liquid nitrogen, gold-coated, and observed.

2.4 Differential scanning calorimetry

Differential scanning calorimetry was performed using a *Perkin-Elmer DSC 7* calorimeter. Samples of trilaurin plus 0.1, 0.2, 0.5, and 1 weight-% phosphatidyl ethanolamine or phosphatidyl choline were prepared. Samples were cooled from 60 °C to –20 °C at 3 °C per min.

2.5 Laboratory-scale fractionation

Simple laboratory-scale dry fractionation was performed on selected systems of palm oil plus 0.2 weight-% phospholipid. Samples of 100 ml of oil were prepared. They were then heated to 60 °C and cooled in air to 20 °C. At this temperature palm oil has a relatively low solid content of about 12% and so a dispersion of liquid and solid was formed. The samples were then filtered under vacuum and the yields of olein and stearin measured.

3 Results

3.1 Initial screening

After the screening procedure the control sample of palm oil was apparently solid and homogeneous (despite a calculated solid content of only about 12%). Samples containing phosphatidyl ethanolamine showed obvious differences with much larger spherulites. At a concentration of 0.5% phosphatidyl ethanolamine liquid oil could be easily poured off. This was not possible at all with the control sample.

The microstructure of the control sample is illustrated in Fig. 1. It consists of small spiky spherulites that interlock to form a net-like structure. Individual crystals are long and very thin (needle-like), are heavily twinned and are of varying length. This gives the appearance of the ragged-looking spherulites.

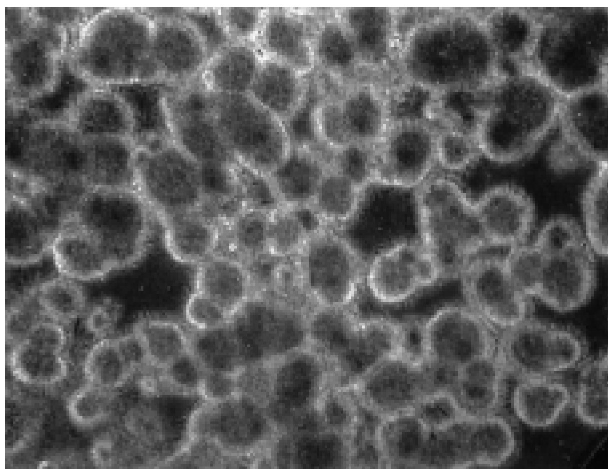

Fig. 1. Spherulites of palm oil.

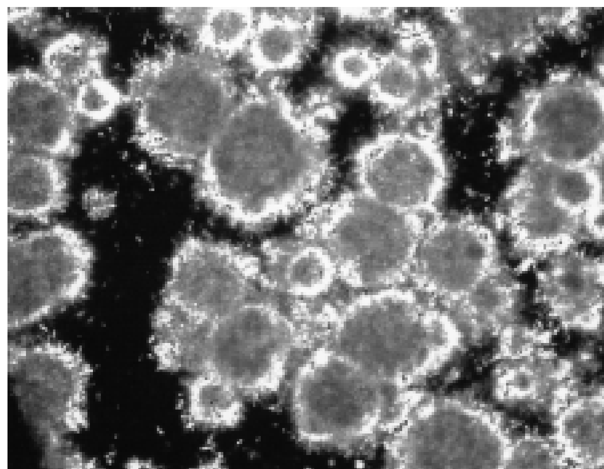

Fig. 2. Spherulites of palm oil plus 0.2 weight-% phosphatidyl ethanolamine.

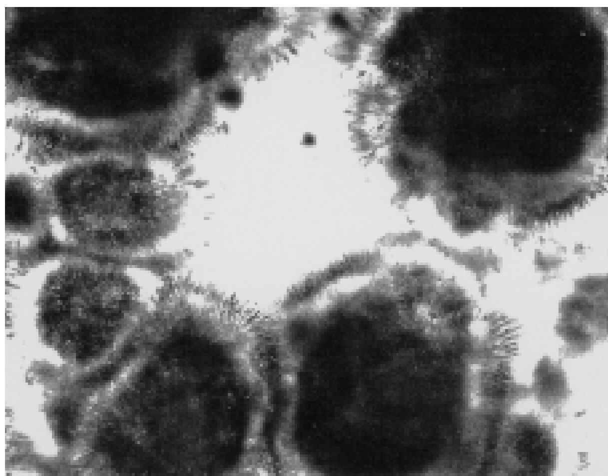

Fig. 3. Spherulites of palm oil plus 0.2 weight-% phosphatidyl choline.

Samples containing phosphatidyl ethanolamine, phosphatidyl inositol and lysophosphatidyl ethanolamine had similar structure to each other. Much larger spherulites were observed which were more uniform in size (Fig. 2). The spiky nature of the spherulites was largely reduced and the interlocking net-like structure was much diminished. The presence of the larger spherulites indicates a retardation effect on the nucleation, whereas the change in the structure shows a modification effect on growth and crystal habit.

The phosphatidyl choline containing samples also gave larger spherulites than the control (Fig. 3), however, in this case there is no change to the spherulitic structure or the shape or size of the individual crystals. This suggests that the phosphatidyl choline is acting in a different manner to the other investigated compounds. There is still an effect on the nucleation, but very little effect on crystal growth.

Scanning electron microscopy (SEM) pictures of the samples confirm these observations (Fig. 4). They show that the structure of the spherulites is significantly altered by the addition of phosphatidyl ethanolamine. A very clear change to the crystal structure is illustrated, as well as the obvious change in crystal size.

3.2 Differential scanning calorimetry

In general additives retarded the crystallisation transformation with phosphatidyl ethanolamine having a much larger effect than phosphatidyl choline (Fig. 5). This showed that the additives are inhibiting the crystallisation of the triglycerides, with additives that are having a larger effect on the crystal habit again here having a larger effect.

A further series of experiments was performed with the addition of synthetic phosphatidyl choline of varying side-chain length from C_{12} to C_{18}. It was found that they all reduced the nucleation and crystallisation rates with an increasing effect with chain length and so the palmitoyl (C_{16}) and stearoyl (C_{18}) compounds had the greatest effect. This shows that chain length is an important factor for considering the effects of an additive and suggests that matching or near matching of chain length gives larger interaction and effect.

3.3 Temperature gradient microscopy

Comparison between trilaurin and trilaurin plus PE samples are shown in Fig. 6. The addition of phosphatidyl ethanolamine caused certain changes to crystal habit. In particular the crystals' aspect ratios are increased and

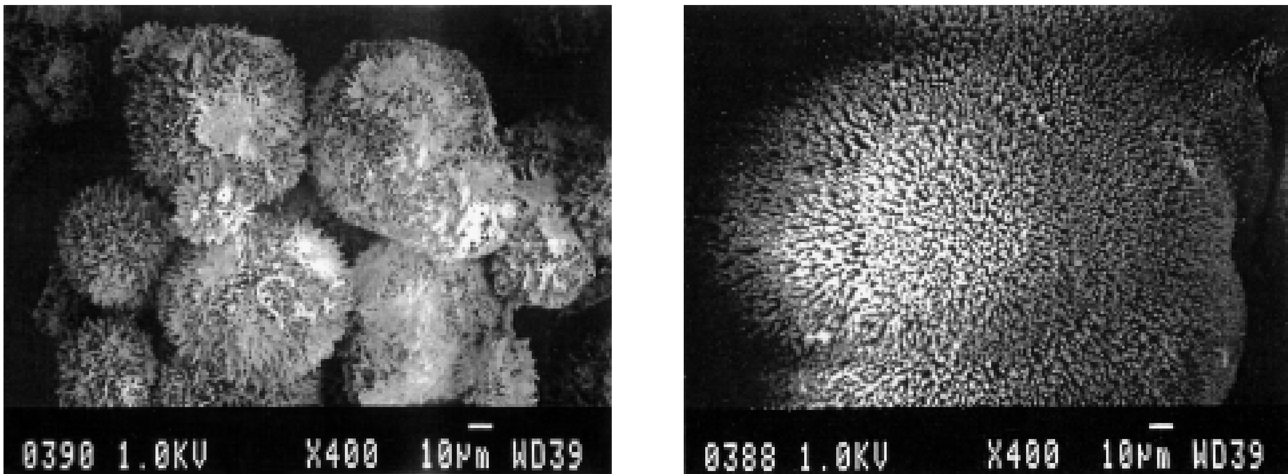

Fig. 4. (a) SEM micrograph of palm oil spherulites, (b) SEM micrograph of spherulites of palm oil plus 0.2 weight-% phosphatidyl ethanolamine.

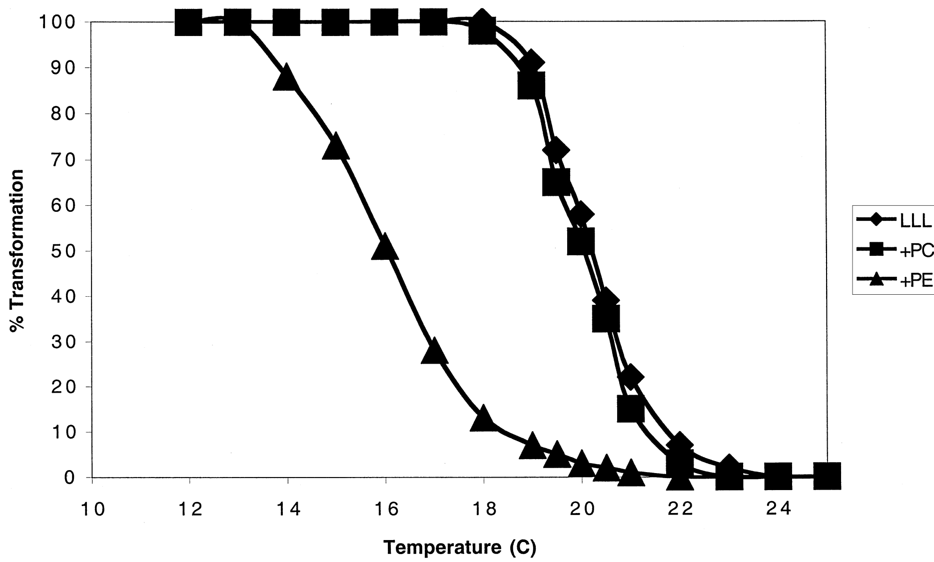

Fig. 5. Solid content vs temperature on DSC cooling of trilaurin (LLL), trilaurin plus 0.2% phosphatidyl choline (+PC) and trilaurin plus 0.2% phosphatidyl ethanolamine (+PE).

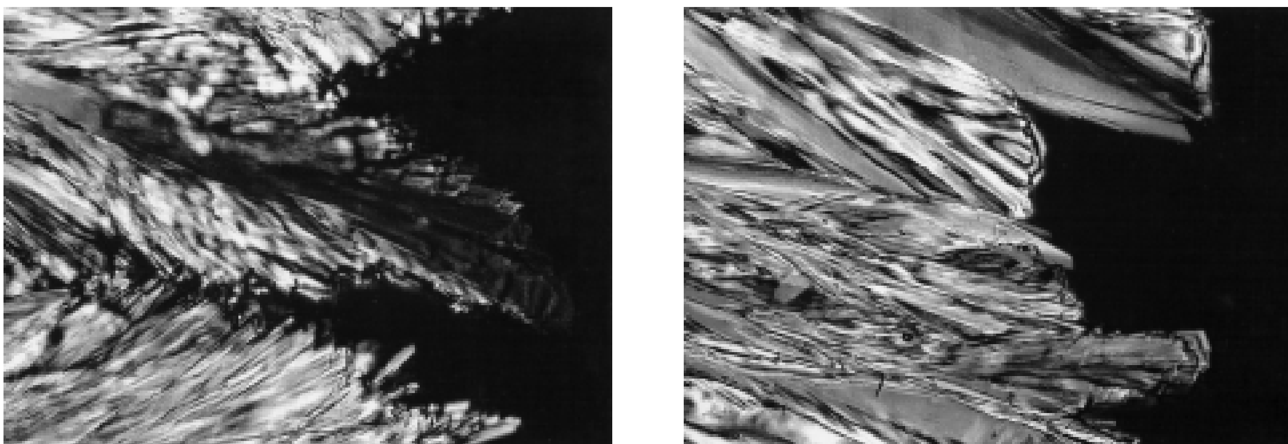

Fig. 6. Micrographs taken on the temperature gradient microscope stage. (a) Trilaurin, (b) Trilaurin plus 0.2% phosphatidyl ethanolamine.

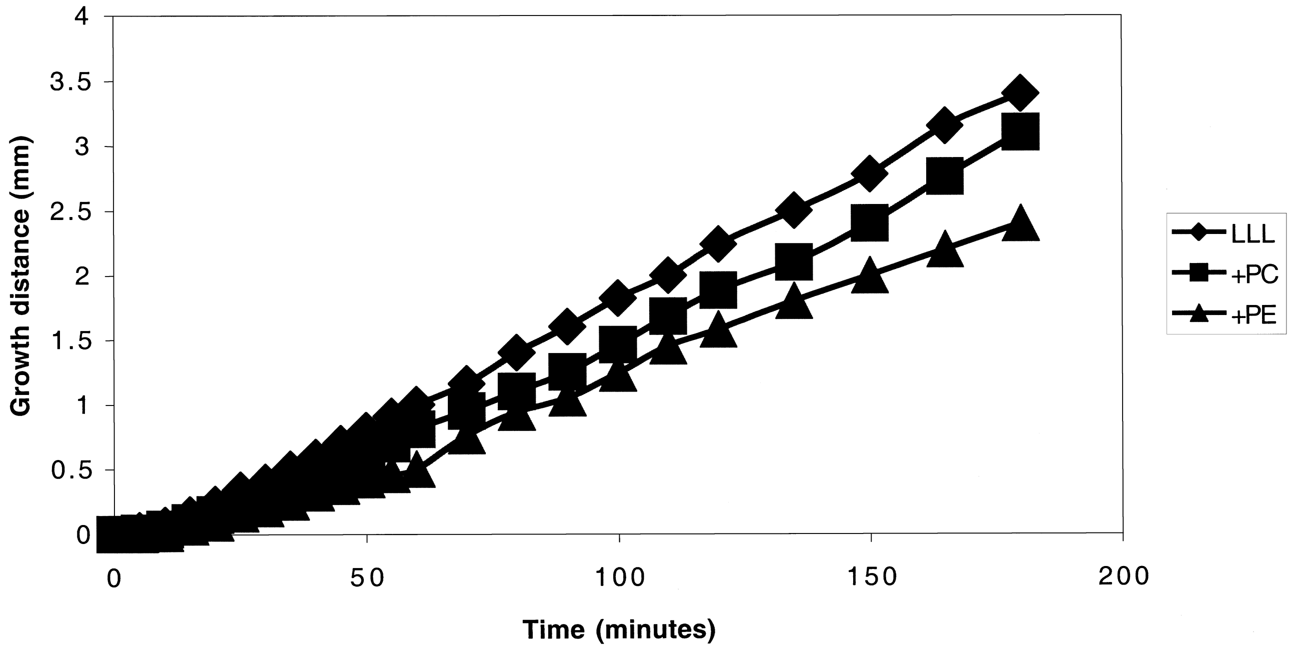

Fig. 7. Growth of crystals of trilaurin (LLL), trilaurin plus phosphatidyl choline (+PC) and trilaurin plus phosphatidyl ethanolamine (+PE) measured on the temperature gradient microscope stage.

there is variation to the characteristic interfacial angles. This shows a large classical effect on the relative growth of the different faces.

Crystal growth rates were measured (Fig. 7). It is clearly shown that the phospholipids retard growth in the fastest growing direction. This indicates that the phospholipids have strong effects on crystal growth as well as any effects that they have on crystal nucleation. Also, different additives have different effects. Therefore, the exact mechanism depends upon the interaction between the particular additive and the growing triglyceride crystals.

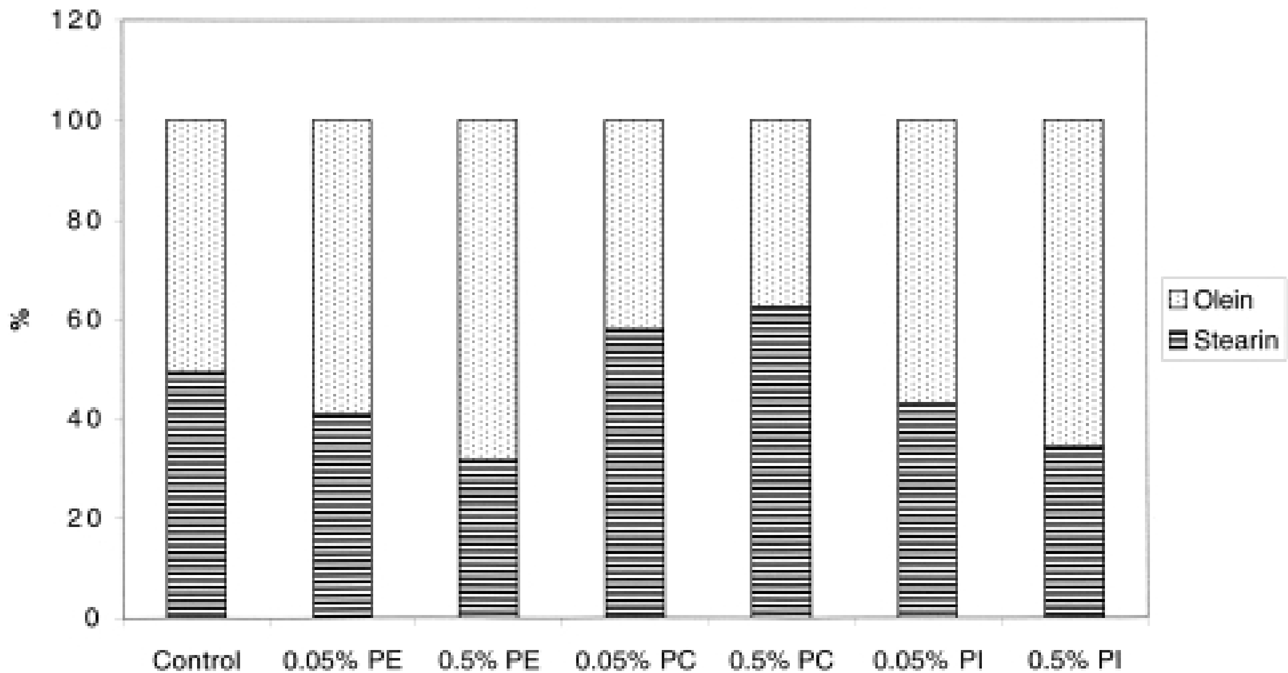

Fig. 8. Yields from the laboratory scale dry fractionation experiments. Samples contain on added phospholipid (control), phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), or phosphatidyl inositol (PI).

3.4 Laboratory-scale fractionation

Laboratory-scale fractionations were performed on certain systems. In particular systems containing phosphatidyl ethanolamine and phosphatidyl choline were studied. Yields are given in Fig. 8. As the real solid content is unchanged from one sample to the next any reduction in the "stearin" apparent solid content is due to less entrapped oil in the solid. Therefore, a higher separation efficiency is indicated. In this case it can be seen that phosphatidyl choline has a negligible effect on the separation efficiency, whereas phosphatidyl ethanolamine improves the separation efficiency by a large amount.

4 Discussion

The results demonstrate how minor components can have a significant effect on the crystallisation behaviour of triglycerides. They show how the addition of controlled quantities of additives can lead to changes in the structure of crystals and so alter the crystal network of a particular system. As a result significant changes can be made in the efficiency of a process or in the behaviour of a product. This technology has been investigated for fractionation application to destroy fat crystal structures and optimise fractionation yields [14, 15]. However, by understanding the mechanisms that are occurring it should be possible to increase network strength and so produce products which have stronger networks. This may be useful to entrap oils in a fat crystal network at a lower content of fat than is possible without crystal habit control.

5 Conclusions

Interactions between additives and triglycerides can significantly alter the shape and size of the fat crystals. This can then have a large effect on the properties of any fat crystal network and thus on the properties of a system (e. g. food product). By control of the interaction different effects are possible. Further work is required in order to relate the mechanisms of the triglycerides: additive interactions to the resultant behaviour of final systems.

Acknowledgements

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