Influence of Phospholipids on the Crystallization of Waxes in Sunflowerseed Oil

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The effect of lecithins on wax crystallization in sunflowerseed oil was studied. The results showed that the presence of lecithins does not modify the nucleation stage, but decreases the crystalline size. Lecithin has a minimal effect on viscosity and does not change the wax/oil equilibrium ratio. It is proposed that the main mechanism of action of lecithin involves the partial adsorption on the surface of the wax crystals interfering with their growth.

Size and morphology exhibited by wax crystals present in sunflowerseed oil during the winterization step depend not only on the variables related to cooling conditions (temperature of the refrigerating fluid, residence time, exchange conditions, etc.), but also on the presence of "impurities" in the oil, which act by inhibiting or delaying wax crystallization (1-4).

According to Rac (2), the presence of 1% lecithin in a refined oil sample containing 1% wax causes a decrease of about 60% in the crystal size in comparison with that obtained in phospholipid-free oils. This author suggested that mucilages would form a protective coating around the wax crystals, thus preventing their growth and agglomeration. It also has been reported that phospholipids act as inhibitors of wax crystallization in cottonseed oil (1,3).

The objectives of the present study are to analyze the effect of lecithins on wax crystallization in sunflowerseed oil and to obtain information about the mechanism of action of these phospholipids.

MATERIALS AND METHODS

Samples. All experiments were performed with refined sunflowerseed oil containing 0.005% wax and 12 ppm phosphorus (lecithin) to which known amounts of waxes and soy lecithins were added. Wax concentration was determined by weighing and ranged from 0.05% to 1%. Lecithin as expressed in terms of phosphorus (5) was measured by standard AOCS methods (6) and ranged from 12 ppm to 600 ppm. Wax used in the experiments was obtained by filtering crude sunflowerseed oil through a Buchner funnel. The solid residue was washed several times with petroleum ether at 0 C and dried in a desiccator at room temperature.

Nucleation. The influence of lecithin on wax nucleation in sunflowerseed oil was studied on oil samples containing different amounts of phospholipids. Samples were melted at 65 C and were then exponentially cooled on the platform of a Leitz microscope as described previously (7). Thermal histories were characterized in terms of the parameter K (a constant which represents the easiness of the heat transfer between the sample and the cooling medium). T_f (temperature of the refrigerating fluid) and T_i (initial temperature of the sample). The temperatures and times of appearance of crystals were determined. The smallest detectable size of crystals was about 1 μm.

Crystal size. Crystal size and morphology were determined from photographs taken one hour after crystallization had occurred. Crystal size was arbitrarily considered as its longest dimension.

Viscosity determinations. Viscosity determinations were performed on samples of refined oil with different lecithin contents (12,48,96,192,288 and 411 ppm of P). A Haake Rotavisco RV2 viscosimeter and NV standard rotor were used; speeds were 90.5,128,181,256 and 362 rpm. Measurements were performed at 20 C.

Conditions of wax/oil equilibrium. Conditions of equilibrium between wax and sunflowerseed oil with high lecithin contents were determined as previously described (7).

Adsorption experiments. Isolated waxes (0.15%) and different amounts of soy lecithin (from 13.4 ppm to 196.9 ppm of P) were added to samples of refined oil (0.005% wax, 12 ppm P). Samples were melted at 80 C and later were linearly cooled at rates of either 30 C/min or 0.1 C/min. After one hour the wax crystals were separated by centrifuging the oil (20,400 $\times$ g for 20 min) at room temperature (T = 15 C). The content of phosphorus remaining in the supernatant oil was then determined (5,6). In all cases the oil obtained after centrifugation was free of wax crystals.

RESULTS AND DISCUSSION

Melting temperatures for various contents of wax and lecithin were determined. No differences due to the presence of lecithin were detected.

Nucleation. Time of appearance of crystals was taken as that elapsing from the time at which the equilibrium temperature was reached (for 0.15% wax, T_e = 50 C) to the time at which the first crystals appeared (corresponds to the time during which the sample remains supercooled).

Under the same cooling conditions, lecithin concentrations from 13.9 to 613.2 ppm P gave the same nucleation times (250-260 sec) with no significant change in the number of crystals/cc. This suggests that phospholipids do not modify the nucleation step. Similar conclusions were reached in similar assays performed with a lower wax content (0.005%).

Crystalline growth. Figure 1 shows the effect of the lecithin content on the crystal distribution as determined one hour after the beginning of crystallization (7). A shift toward smaller sizes is observed with increasing lecithin contents.

Figure 2 shows the mode ($\bar{D}$) of the crystalline distributions obtained under the same crystallization conditions as a function of the lecithin content of the sample and for different wax contents. The results

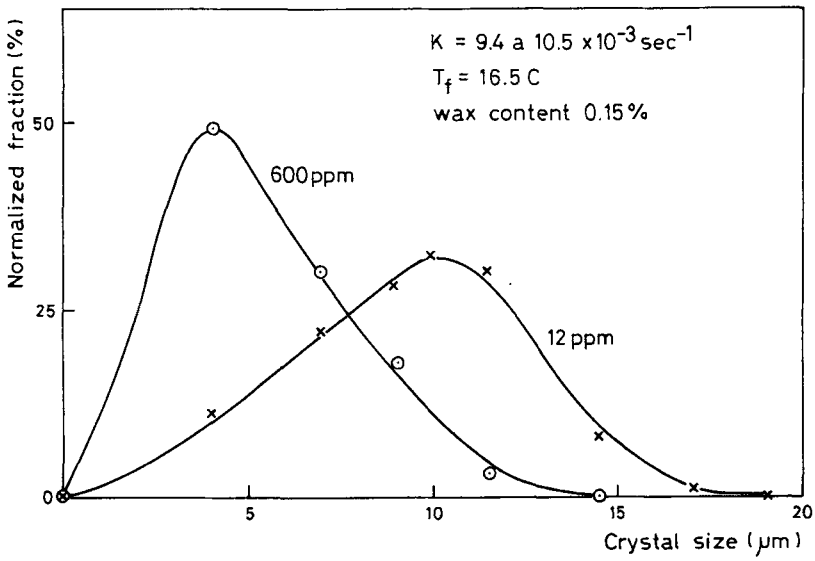

FIG. 1. Crystal size distributions.

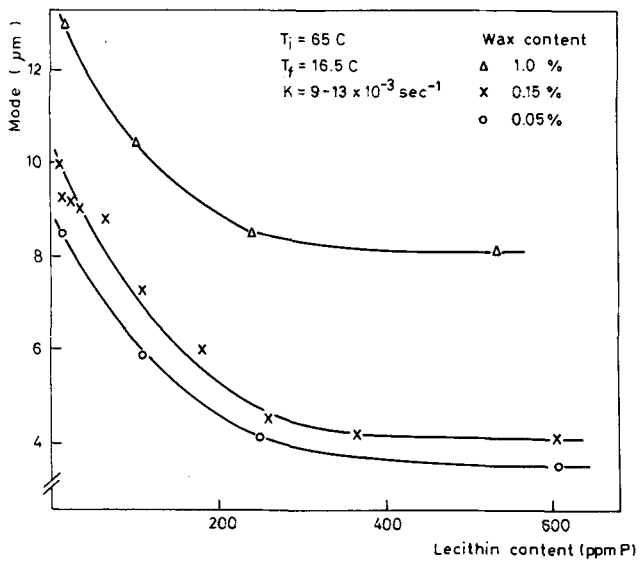

FIG. 2. Effect of lecithin content on the crystalline size for samples with different amounts of waxes.

obtained indicate that the crystalline size decreased to about one-half by adding 300 ppm phospholipids (expressed as P) in agreement with previous reports (2). At higher concentrations the mode becomes virtually independent of the lecithin content.

Experiments performed with different wax concentrations showed that the inhibitory effect was smaller at higher wax concentrations. In Table 1 this effect is expressed as the percent decrease of the mode 100 $(\bar{D}_o - \bar{D})/\bar{D}_o$ with reference to the value obtained for lecithin-free oil (12 ppm P), where $\bar{D}_o$ is the mode of crystal distribution at the above mentioned concentration. Table 1 shows that for a constant wax content there is an increase of the inhibition at increasing lecithin concentrations until a maximum value of near 60% is attained. A similar effect is observed when the percentage of wax is reduced while the amount of lecithin is kept constant. It should be remembered that when the amount of wax in the oil is reduced, the

TABLE 1

Effect of the Lecithin Content on Wax Crystals Growth in Sunflowerseed Oil

Reduction of the percentage of crystal size 100 $(\bar{D}_o-\bar{D})/\bar{D}_o$			
Lecithin content (ppm P)	Wax concentration (%)		
	1.0	0.15	0.05
96	20	28	30
240	35	53	53
384	38	58	56

number of crystals also decreases under the same cooling conditions; thus, in order to obtain an equivalent inhibitory effect a lower amount of lecithin is required as the wax concentration in the oil decreases.

Figure 3 shows the variation in size of a crystal corresponding to the largest fraction in the distribution as a function of the growth time for lecithin concentrations ranging from 12 to 600 ppm of P. As can be seen, the presence of phospholipids decreases not only the final size attained by the crystal, but also its growth rate. From the shape of the curves obtained, it is observed that the inhibitory effect is present from the beginning of growth, suggesting a permanent interference of lecithin with incorporation of waxes to the original crystal.

Possible mechanisms of action of lecithin. It has been found that lecithin has a minimal effect on viscosity, which increases only 3% when the lecithin content is raised from 12 ppm (P) to 411 ppm (P). This fact, together with the above mentioned results and the absence of changes of the wax/oil equilibrium ratio, led to the idea that the main mechanism of action of lecithin could involve its partial adsorption on the surface of the wax crystals, thus interfering with their growth. Therefore, an experiment of crystallization of waxes in the presence of lecithin was performed, and the phosphorus remaining in the oil after the separation of crystals was determined as

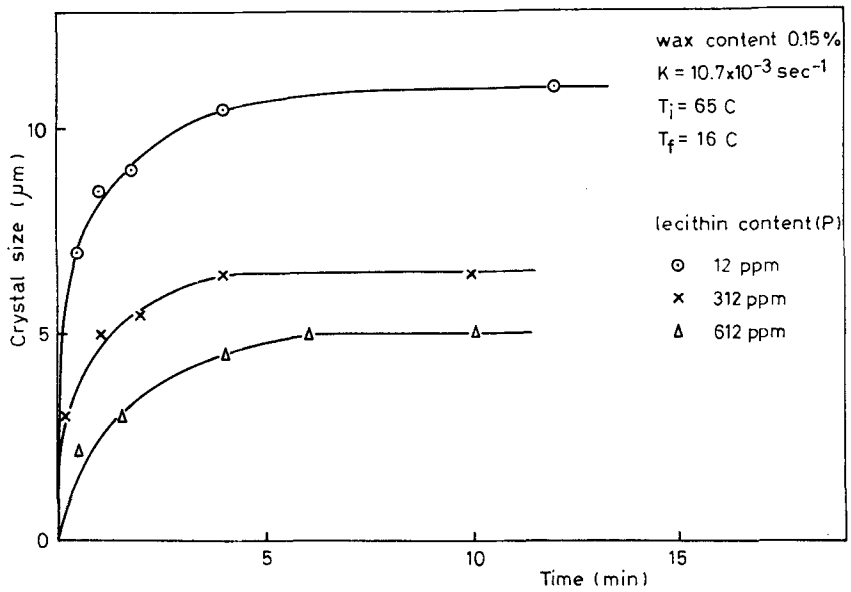

FIG. 3. Crystal size as a function of time for samples with different contents of phospholipids.

described under Materials and Methods. This experiment was also performed at two rates of cooling, in order to obtain different numbers and sizes of crystals.

Figure 4 shows the percentage of lecithin retained by the crystals for different initial contents of lecithin. It can be seen that the percentage retained becomes higher for higher initial concentrations of lecithin, in agreement with the adsorption mechanism proposed.

In other words, the availability of lecithin for incorporation to the crystal is related to its concentration in the solution. Lecithin molecules would compete with those of wax for equivalent sites on the crystal, thus originating an inhibitory effect which delays the crystal growth rate, as can be seen in Figure 3. It is important to take into account in Figure 4 that under the same cooling conditions, the number of crystals is the same in all the experiments. However, because the inhibitory effect is higher at higher lecithin concentrations (Fig. 2), the amount of wax which crystallizes is not constant for the experiments described in Figure 4. This would indicate that the percentages retained per mass unit of crystallized wax would increase more markedly than is shown in Figure 4. It also shows a little difference among the amounts of phospholipids adsorbed at different cooling rates. This effect may be interpreted to mean that in cylindrical crystals the main contribution to the total surface corresponds to the lateral area. Considering that the cooling rate principally modifies the number and length of crystals with little change in the diameters, the ratio of the areas presents a value close to the adsorption ratio shown in Figure 4.

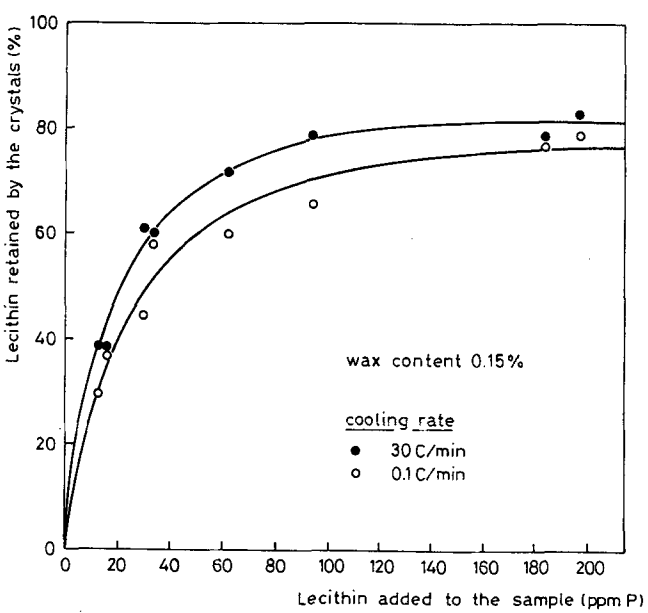

FIG. 4. Lecithin retained by crystals as a function of the amount of lecithin added to the sample.

It is concluded that phospholipids affect the growth of wax crystals by partial adsorption on their surface, interfering with the incorporation of new molecules on the crystals. After a certain time, phospholipids totally cover the surface, preventing the incorporation of waxes. The crystals formed are smaller, and the waxes remaining in the solution are greater than those present during the crystallization without phospholipids.

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