# PHASE EQUILIBRIA AND STRUCTURES IN THE AQUEOUS SYSTEM OF WHEAT LIPIDS

T. CARLSON, K. LARSSON, and Y. MIEZIS, Department of Food Science, University of Lund, Box 740, 220 07 Lund 7, Sweden

Cereal Chem. 55(2): 168-179

**ABSTRACT**

The phase behavior of the aqueous system of lipids extracted from wheat flour (Amy) is described. The phase relations are illustrated in a ternary phase diagram water/polar lipids/nonpolar lipids. The structures have been characterized by X-ray diffraction. At low water content [up to about 15% (w/w)] a liquid crystalline phase of inversed hexagonal type is formed, and at a water content of about 15–50% (w/w) a lamellar liquid crystalline phase exists. A unique feature in this system is the formation of a liquid lipid-water phase of so-called L2-type. It contains large amounts of water [about 75% (w/w)]. It is proposed that the structure is related to the lamellar liquid crystalline phase by a melting process so that only the long-range order is lost. In addition to these three lipid-water phases there also exists an oil phase (nonpolar lipids) and a water phase in large areas of the phase diagram. The functionality of wheat lipids is discussed in relation to the phase properties.

Wheat-flour contains about 2% (w/w) lipids which are known to have a significant influence on the breadmaking process by interaction with the protein and starch components of the flour (1,2). Numerous studies concern this role of the wheat-flour lipids, often based on lipid extraction of dough and flour (2) or by reconstitution of various extracted lipid fractions with the defatted (or delipidized) flour (3). Knowledge on the structures formed by interaction of the wheat lipids with water is lacking, and the present work concerns the phase behavior and physical structures in the aqueous systems of extracted wheat lipids. From a physical point of view the wheat lipids like all natural lipid mixtures can be separated into a nonpolar fraction, defined as components with no interaction with water, and a polar fraction containing components, which form association structures with water. It is therefore convenient to represent the phase behavior of wheat lipids-water by a ternary system, where the polar and nonpolar components form two of the corners. The structures of the liquid-crystalline phases formed by the interaction of polar lipids with water were first derived by Luzzati and co-workers (4), and the use of ternary phase diagram of this type has been used particularly by Ekwall and co-workers (5). The significance of these properties in food systems has also recently been reviewed (6).

Electron microscopy studies of flour (7) seem to indicate that the lipids of predominatly polar type occur in membrane-like aggregates, whereas the lipids of mainly nonpolar type form aggregates resembling oil droplets (8).

The ternary system presented below gives information on possible structures formed when the lipid aggregates in the flour interact with water. The different types of lipid aggregates correspond to different polar/nonpolar ratios in the ternary phase diagram. Knowledge of these ratios and of the amount of water in the dough available for lipid interaction are, however, needed in order to

0009-0352/78/000021$03.00/0

understand the physical structure of the lipids in the dough and how they interact with proteins and starch.

## MATERIALS AND METHODS

An untreated sample of a spring wheat, Amy, from the 1975 crop was experimentally milled in Brabender Quadromat Senior with approximately 65% extraction. The flour contained 13.5% (w/w) proteins ($N \times 5.7$), less than 0.5% ash, and 1.9% crude fat, all calculated on a dry basis. All organic solvents were analytical reagent grades, and solutions were prepared from analytical reagent grade compounds. The reference lipids used were commercial diplamitoyllecithin and lysolecithin (Sigma) and a synthetic monogalactosyl diglycerides (stearic acid) kindly supplied by the chemical synthesis service of the Swedish Natural Science Research Council. Protein, moisture contents, ash, and crude fat were determined as described in AACC methods 46-11, 44-40, 08-03, and 30-10 (9).

Flour and water saturated *n*-butanol (WSB, volume ratio 20:80) in the ratio of 1:4 (w/w) were mechanically stirred for 150 min at 20° C. The extract was then centrifuged at $1800 \times g$ for 60 min. The supernatant was collected and solvents were removed in a rotating evaporator under vacuum below 45° C. Ethyl-ether was used to dissolve the lipids in the residue and the ether was evaporated using a stream of nitrogen. The lipid residue was finally dried over $P_2O_5$ for 24 hr and stored at −18° C. The amount of lipids was determined gravimetrically.

The crude lipid mixture obtained by extraction was separated according to MacMurray and Morrison (10) into nonpolar and polar lipids. A simpler and faster method (11) was used in order to obtain polar lipids in amounts sufficient for phase studies. The method was modified with a fourth eluation step using acetone to obtain monogalactosyl diglyceride, as the method originally (11) was developed for separation of phospholipids.

Thin-layer chromatography (tlc) analysis was done using plates with 0.25 mm silica gel (Merck). The solvent system was chloroform:methanol:water 65:25:4 (v/v/v) and the plates were sprayed with a solution of 30.0 g copper acetate in 92.2 g 85% phosphoric acid and heated for 30 min at 180° C.

The lipid-water phases were prepared by mixing water and the extracted lipids in ratios differing by 5 or 10% (w/w) in composition. Each sample was mixed and kept at room temperature during about 24 hr in order to reach equilibrium, and then centrifuged at $40,000 \times g$ for 2 hr. The water content of the phases was determined by the Karl-Fischer method. The different phases were then examined in the polarizing microscope and characterized by X-ray low-angle diffraction using a Guiner type of camera, as described by Larsson (12).

## RESULTS AND DISCUSSION

### Lipid Separation

Eighty-eight percent of the crude fat was extractable with WSB. Of this lipid mixture 55% (w/w) were polar lipids and 45% were nonpolar separated as described by MacMurray and Morrison (10). In the faster separation method (11) the first and the fourth eluates were taken as polar lipids and the second and the third as nonpolar lipids. The method by MacMurray and Morrison (10) is

based on eluation by hexane/ethyl ether/ether/chloroform/acetone/methanol, whereas the method by Sen Gupta (11) uses light petroleum/ethyl ether/acetone. The ratio polar/nonpolar lipids obtained in this way differed by less than 1% from values obtained by the method by MacMurray and Morrison (10) (*i.e.*, below the experimental errors). The overall recovery from the column was 98%. A tlc-pattern shown in Fig. 1 illustrates the resolution of this simpler separation method.

A small amount of protein, which also is obtained by this lipid extraction, appeared to have no influence on the phase properties. Protein and contaminating starch granules can be separated by ultracentrifugation.

**Phase Behavior**

The phase diagram of the ternary system polar wheat lipids, nonpolar wheat lipids and water is shown in Fig. 2. Different proportions of polar/nonpolar lipids obtained by chromatographic separation or by separation of well-defined phases of Fig. 2 were mixed with water, and the phases formed were analyzed as described above. At all compositions above 5% (w/w) of water a liquid oil phase, free from polar lipid components according to tlc, is formed together with other

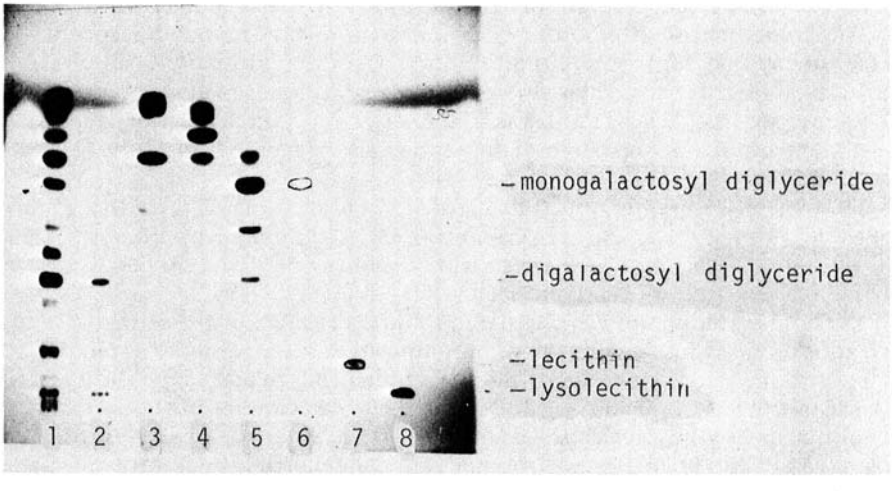


Fig. 1. TLC-pattern of the liquid phase L2 containing about 10% (w/w) nonpolar and 90% polar lipids. From left to right is shown the total lipids, the first fraction in light petroleum (40°–60° C), the second fraction in light petroleum - ethyl ether 87:13 (v/v), the third fraction in ethyl ether, the fourth fraction in acetone, and the standards monogalactosyl distearin, dipalmitoyl lecithin and lysopalmitoyl lecithin. The solvent system used was chloroform-methanol-water (65:25:4). The spots from the reference lipids are weak due to their saturated character and have therefore been indicated by black pencil. The first fraction is dominated by digalactosyl diglycerides, the second by triglycerides and fatty acids, the third by sterols and the fourth by monogalactosyl diglycerides.

phases. Four types of phases dominate the phase diagram. Besides an oil and a water phase, two types of lipid-water phases are formed. One is a liquid phase which has the characteristics of a so-called L2-phase (5) and the other is of liquid/crystalline character (4). As shown in Fig. 2 the extracted lipids have a polor/nonpolar ratio of 55:45 and the broken line from this point to the water corner represent the binary system formed by wheat lipids and water.

All compositions of water added to total extracted lipids caused a nonpolar oil phase to separate on top of the samples. At low-water content a hexagonal liquid crystalline phase is formed, which at higher water content is transformed into the lamellar liquid crystalline phase. The structure of the two liquid-crystalline phase is illustrated in Fig. 3.

The hexagonal spacings, equal to the distance between the center of the water cylinders, were found to be 70.4 and 73.2 Å when water was added to the total

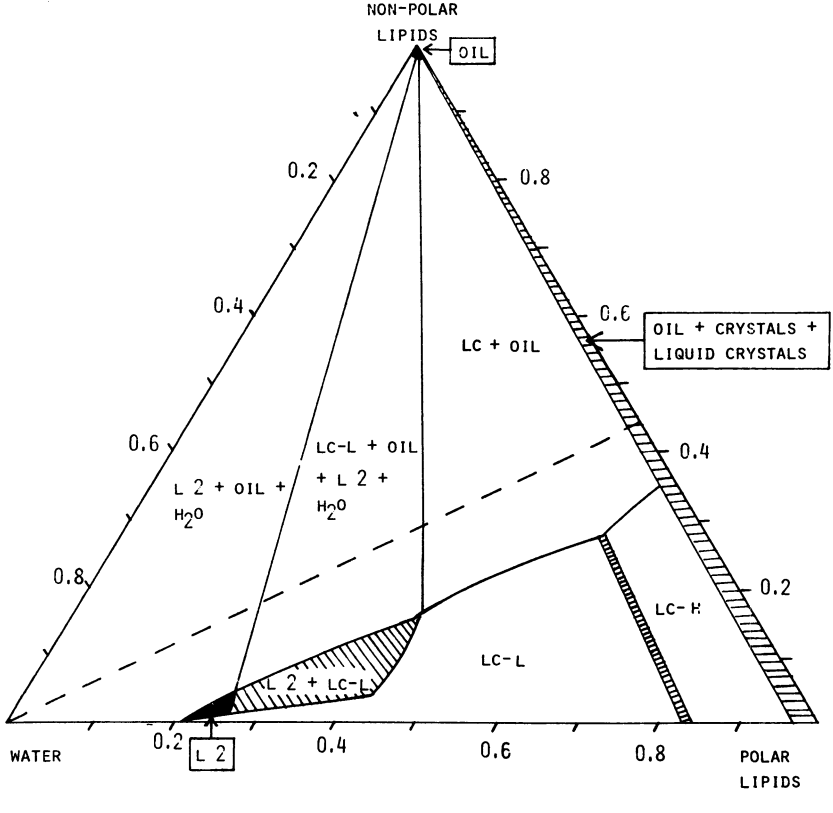


Fig. 2. Ternary phase diagram of wheat lipids and water at 25° C represented by the polar and nonpolar lipid fractions as two of the components. The hexagonal and lamellar liquid crystalline phases are called LC-H and LC-L, respectively. The broken line corresponds to the binary system of the total extracted lipids and water.

lipids to a water content of 6 and 12% (w/w), respectively. These parameters are reasonable in comparison with the lamellar spacings given below.

The lamellar liquid crystalline phase exists over a wide composition range, and at high water content there is a successive transition to a liquid phase (L2) described below. The swelling properties of the lamellar liquid crystalline phase is shown in Fig. 4. When this phase is formed by the total extracted lipids and water, the thickness of the lipid bilayer (obtained by extrapolation to a water content of zero) is 57 Å. The ratio of nonpolar/polar lipids in this phase is about 35:65 (w/w). When the lamellar phase is enriched into polar lipids to a ratio nonpolar/polar of about 20:80, the repetition period of the lamellar phase is reduced, and the lipid bilayer thickness is 49 Å. The spacings of the lamellar phase formed by only polar lipids correspond to a lipid bilayer thickness of 40 Å. These differences show that the triglyceride molecules of the nonpolar lipids solubilized in this phase, to a large extent must be accommodated in the gap between the lipid bilayer, and not arranged with their chains along the chains of the polar lipid molecules. In the case of lipid fractions with a nonpolar/polar ratio of 20:80 it was possible to record sharp diffraction lines corresponding to spacings up to 124 Å. The lamellar liquid crystalline phase exists up to very high water contents but the diffraction lines are successively broadened and hard to measure due to reduced domain size of this phase, which is related to the presence of the liquid phase (L2), as will be further described.

Relative to this, the aqueous system of galactolipids has been studied by Shipley *et al.* (13), who found that a monogalactosyl diglyceride forms only a hexagonal liquid-crystalline phase with water of the same type as described here, whereas the corresponding digalactosyl diglyceride forms a lamellar liquid-crystalline phase. They both exist in the composition range of about 10–20% (w/w) of water. It is also known that various phospholipids form a lamellar liquid-crystalline phase with water (4), whereas there are no studies of mixed

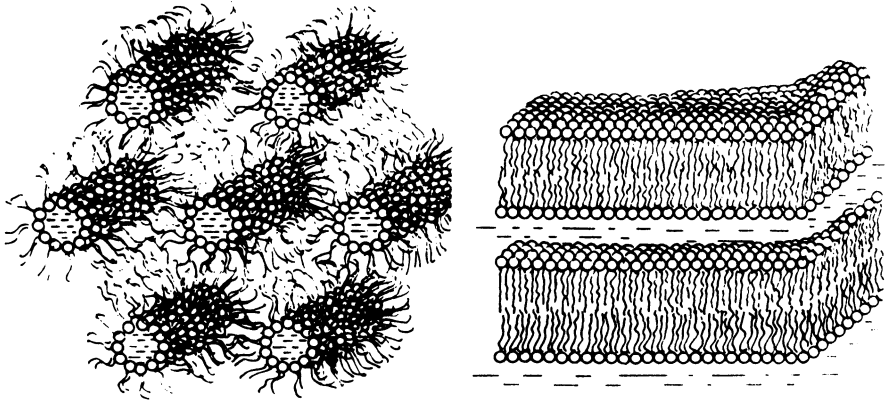

Fig. 3. Illustration of the structure of the lamellar liquid crystalline phase (left) and the hexagonal liquid crystalline phase (right) occurring in aqueous systems of wheat lipids.

systems of galactolipids and phospholipids, such as the polar fraction of wheat lipids.

The most remarkable feature of this phase diagram is the existence of an L2-phase formed in excess of water. It is an optically isotropic liquid with the appearance of a clear yellow oil, and contains about 75% (w/w) of water. It exists only in the presence of a water phase, and since it is heavier than water, it is separated from the triglyceride oil phase by a water layer (Fig. 5). The L2-phase of this system is unique in many respects when compared with L2-phases described earlier by other authors, thus it is discussed in detail.

The so-called L2-phase was first described by Fontell and co-workers (14) in the ternary system sodium caprylate:decanol:water as an amphiphile in the liquid state containing water in the form of inversed micelles (L1 is the ordinary micellar water solution). It has later been observed in numerous other amphiphile-water systems (5). The detailed structure is not known. On the basis of an X-ray scattering analysis of this phase in binary system, particularly the swelling in relation to the lamellar liquid crystalline phase, it was proposed that it possesses the same gross structure as liquid crystalline phases, except that the

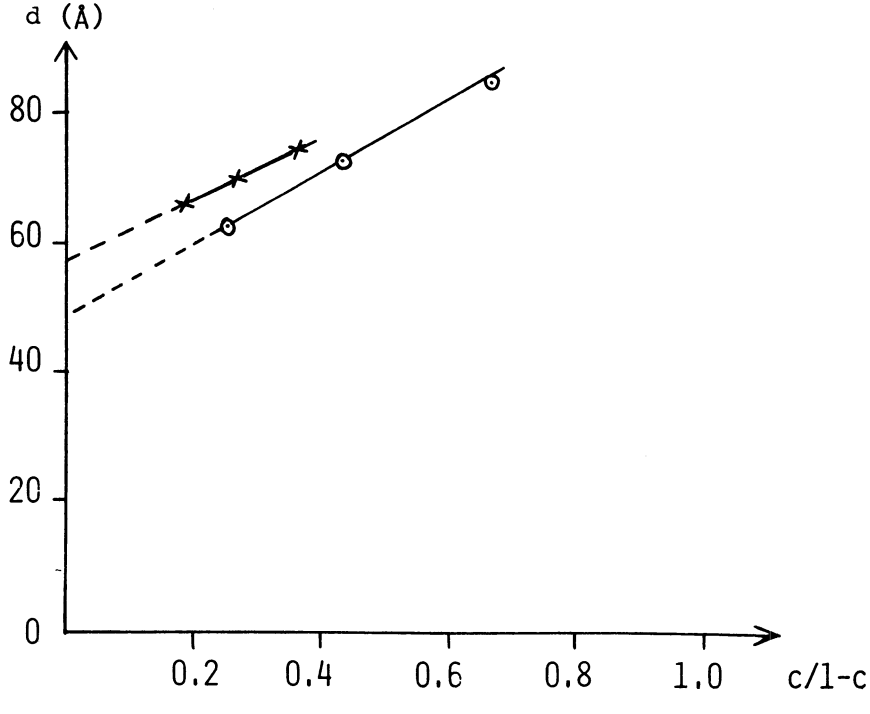


Fig. 4. Lamellar unit distance obtained by X-ray diffraction of the lamellar liquid crystalline phase as a function of $c/L - c$, where $c$ is the weight fraction of water. Crosses represent samples obtained when water is added to the total lipids, and circles correspond to a polar-enriched lipid fraction, where the polar/nonpolar ratio is about 80:20.

long-range order is lost (15).

It is thus formed by melting of the lamellar or inversed hexagonal liquid-crystalline phases, and the X-ray data indicate that the structural units are the same. In the case of an L2-phase formed by melting the lamellar liquid crystal of monoglyceride-water phases, the size of the lamellar units was estimated to be about 300 Å; *i.e.*, water occurs as lamellar disks with this diameter in a lipid environment (12,15). In the present system the L2-phase is formed from the lamellar liquid crystalline phase by heating, and the L2-phase is, therefore, proposed to consist of lamellar units, *i.e.*, the same type of structure as in monoglyceride-water systems (15).

The L2-phase has not previously been observed in aqueous systems of any natural lipids. Its formation in wheat lipids is probably a consequence of disorder due to the high water content, the presence of nonpolar lipids (for the nonpolar/polar lipid ratio of this phase see Fig. 1), and the high degree of unsaturation of hydrocarbon chains (10), so that the 'melting point' of the lamellar liquid crystalline phase is lowered. The amount of lysolecithin in the lipid extract is an important factor for formation of this phase.

According to the general behavior of lipid-water systems, the addition of water to the lamellar liquid crystalline phase above its limit of swelling can result in

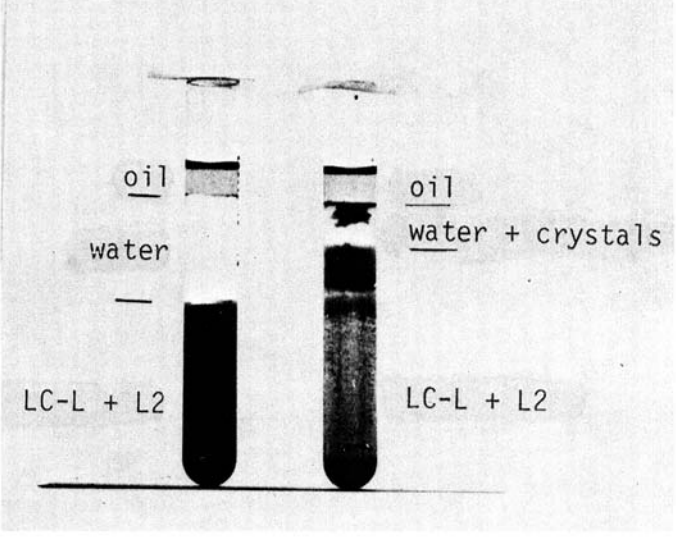


Fig. 5. Samples of lipids from Amy (right) and Maris Huntsman (left) equilibrated with 70% (w/w) of water and separated by ultracentrifugation (300,000 × *g* for 2 hr). Under the oil and water phases there is first a region of lamellar liquid crystals dispersed in the L2-phase (see Fig. 7) and under this region there is a pure L2-phase. Some lipid crystals can also be seen in the water phase formed by Amy lipids, but this is only a consequence of the strong sedimentation gradient.

three alternative phase changes. The first is the formation of a hexagonal phase in the case of lipids with micellar solubility in water, which is not the case in the present system (4). The second and most common is the formation of a dispersion of closed particles with spherically concentric lipid bilayers alternating with water layers, so-called 'liposomes' (12). The third alternative corresponds to temperatures above the 'melting point' of the liposomes, when an L2-phase is formed in equilibrium with water, as observed in the wheat lipid-water system. These phase relations are illustrated in Fig. 6 in the case of two simple aqueous

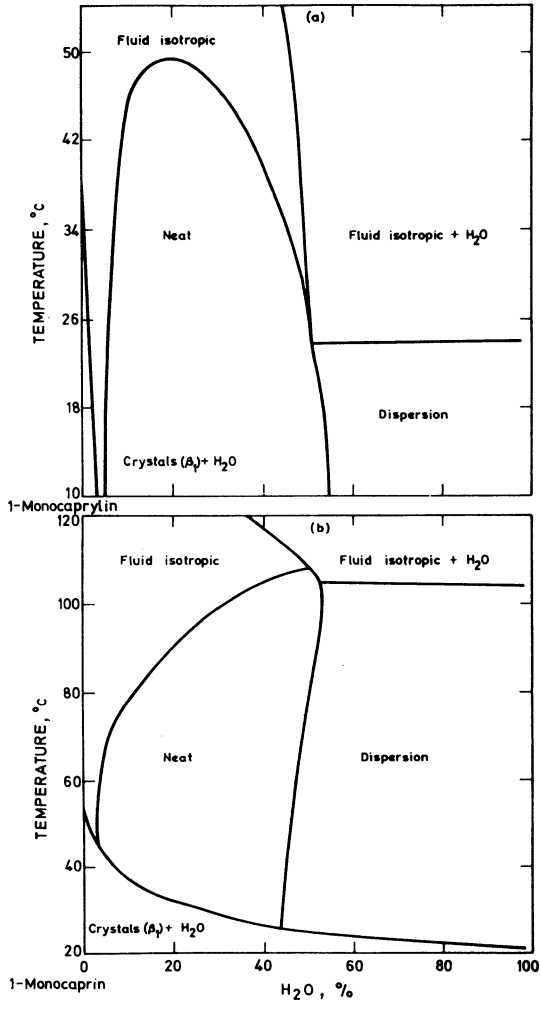


Fig. 6. Binary system of 1-monocaprylin-water and 1-monodecanon-water (12) illustrating the relation between the L2-phase (denoted fluid isotropic in the diagram) and the lamellar liquid crystalline phase.

systems of monoglycerides. Above 24°C 1-monocaprylin forms an L2-phase in equilibrium with water. In the phase diagram of the wheat lipids (Fig. 2) there is a broad region where the L2-phase and the lamellar liquid crystalline phase coexist. This is an anomaly not in agreement with a simple melting behavior, and indicates that there is segregation of the polar lipid molecules so that the ones with higher 'melting point' are enriched in the liquid crystalline phase. This region of the phase diagram is also the only one which gives deviations from the phase rule applied to a three-component system. Thus there is a large four-component triangle (Fig. 2) from this region toward the oil corner, which shows that the system behaves as consisting of at least four components, and the polar lipids are therefore expected to deviate from the simple one-component behavior in this region.

The existence of lamellar phases with a water layer thickness above about 20 Å is due to the presence of charged lipids (4,16). The existence range of the L2-phase with as high water content as observed in the present system should therefore be expected to be sensitive for the amount of charged lipids. It is known that different types of lipids occur in about the same relative amounts in wheat (10), but very small chemical differences, particularly with regard to proportion of ionic lipids, might result in formation of different association structures. We have compared the lipids from the cultivar Maris Huntsman (poorer baking properties than Amy) with those from Amy in order to check this, and the result is shown in Fig. 5. With 70% (w/w) of water added it is obvious by visual comparison that there are pronounced differences. The lipids from Amy give a dominating L2-phase (70% w/w) with water (17% w/w) and nonpolar oil (13% w/w), whereas lipids from Maris Huntsman give a dispersion of liquid crystalline phase in L2, about the same relative amount of oil and about double the relative amount of water.

As suggested the L2-phase can be regarded as a melt, and crystallization is achieved by cooling or by reduction of water content. The crystallization has been studied in the polarizing microscope. The lamellar liquid crystalline phase is always obtained as spherical droplets dispersed in the L2-phase. As seen in Fig. 7 it shows the same optical texture as the dispersion formed by the lamellar liquid crystal in excess of water (12). This shows that the lamellar liquid crystalline phase dispersed in the L2-phase has the same type of closed structure as the 'liposomes', *e.g.*, particles with spherically concentric lipid bilayers alternating with water layers. In order to fit with the suggested structure of the L2-phase, with water disks in a hydrocarbon chain matrix, the surface of the 'liposomes' in the present system should, however, be expected to consist of hydrocarbon chain tails. In this way the interfacial energy between the L2-phase and the liquid-crystalline particles will be extremely low, which should explain why the particles form very stable 'micro dispersions' with no tendency to aggregate and separate, even at centrifugation at 200,000 $\times g$ for 2 hr. This shows also that the density of these two phases must be almost identical, which is consistent with the structural relations proposed here.

X-Ray diffraction studies of lipids extracted from wheat flour have been reported previously by Traub *et al.* (18) and Grosskreutz (19). Differences in these data might be due to different degree of water swelling or even different phases, as the general structural features of lipid-water systems were not known at that time.

**On the Functionality of Wheat Lipids**

Lipids extractable from flour in dough with light petroleum and WSB are classified as 'free' and 'bound', respectively. The term 'free' and 'bound' lipids, based only on extraction behavior, need not have any true relation with the association of the lipids with other flour components.

It has been observed that the amount of 'free' lipids is reduced if the flour is treated with water to give a dough (1). This is not surprising with regard to the phase properties evident from the present work. When water is added, the liquid crystalline phases formed will solubilize nonpolar lipids, and the ordered association structure, like the lamellar phase with hydrophilic interfaces, will protect the nonpolar lipids against nonpolar solvents.

In a recent work on the effect of sucrose esters in breadmaking (20) it was found that the effect increased with the polarity of the lipid, and the best effect was obtained when the HLB-value (hydrophilic/lipophilic balance) was as high as that of diagalactosyl diglycerides.[1] Again, the liquid crystalline phase formed by digalactosyl diglycerides is of lamellar type, where the less polar monogalactosyl diglycerides give a hexagonal liquid crystalline phase.

[1]Recent published work by F. MacRitchie (J. Sci. Food Agr. 28: 53, 1977) supports these findings.

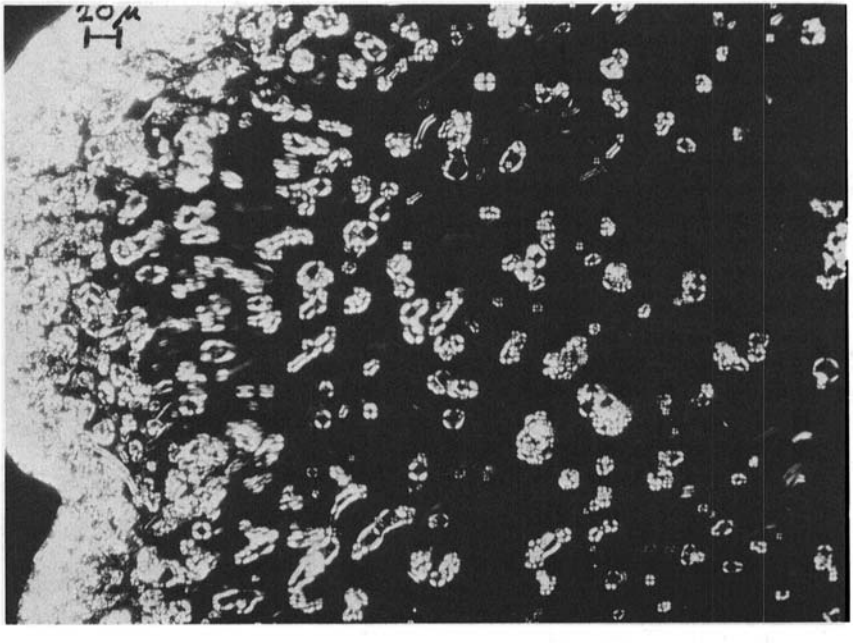


Fig. 7. Formation of the lamellar liquid crystalline phase in the form of closed spherically concentric particles dispersed in the L2-phase as seen in the polarizing microscope. The border to the left is continuous liquid crystalline region.

The lamellar and hexagonal liquid crystalline phases shown in Fig. 3 have quite different rheological properties. The hexagonal phase forms aggregates uninfluenced by excess water or other phases present in the system, whereas the lamellar phase forms multilamellar films at the oil/water, air/water (6) or starch/water (21) interfaces. The L2-phase might also have functional effects. An interesting property of this phase is the liquid state, a unique feature which should be very favorable for diffusion to other components.

The effect of different nonpolar wheat lipids was recently reported (22) by a study of the breadmaking when these lipids were added to flour and defatted flour. Fatty acids were found to give a pronounced negative effect, and of different fatty acids linoleic acid was dominating. A reason for this might be that fatty acids added to a lamellar liquid crystalling phase have a tendency to change the structure to hexagonal. We have found that fatty acids added to lamellar liquid crystalline phases of monoglycerides give hexagonal phases, and that fatty acids are more effective in this respect than triglycerides. This is probably due to a higher tendency of fatty acids to be solubilized in the lipid bilayers of the liquid crystalline phase, whereas triglycerides tend to form a separate oil phase. Furthermore the tendency to form hexagonal phases increases with increasing amount of *cis*-double bounds in the chains.

Phase studies of lipids from other cereals are currently in progress.

**Acknowledgments**

We are grateful to Suwan Poovarodom for assistance with the X-ray work. Financial support has been obtained from the Swedish Board for Technical Development.

**Literature Cited**

1. MECHAM, D. K. Lipids. In: Wheat: Chemistry and technology, ed. by Y. Pomeranz (rev.), p. 393. Amer. Ass. Cereal Chem.: St. Paul, Minn. (1971).
2. POMERANZ, Y. Composition and functionality of wheat flour components. In: Wheat: Chemistry and technology, ed. by Y. Pomeranz (rev.), p. 585. Amer. Ass. Cereal Chem.: St. Paul, Minn. (1971).
3. Mac RITCHIE, F., and GRAS, P. W. The role of flour lipids in baking. Cereal Chem. 50: 292 (1973).
4. LUZZATI, V. X-Ray diffraction studies of lipid-water systems. In: Biological membranes, ed. by D. Chapman, p. 71. Academic Press: New York (1968).
5. EKWALL, P. Composition, properties and structures of liquid crystalline phases of amphiphilic compounds. In: Adv. in Liquid Crystals 1: 1 (1975).
6. FRIBERG, S. (ed.). Food Emulsions. Marcel Dekker: New York (1976).
7. SIMMONDS, D. H. Wheat-grain morphology and its relationship to dough structure. Cereal Chem. 49: 324 (1972).
8. SIMMONDS, D. H. The ultrastructure of the mature wheat endosperm. Cereal Chem. 49: 212 (1972).
9. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Approved methods of the AACC. The Association: St. Paul, Minn. (1976).
10. MacMURRAY, T. A., and MORRISON, W. R. Composition of wheat-flour llpids. J. Sci. Fd. Agric. 21: 520 (1970).
11. SEN GUPTA, A. K. Mizellbildung von Phosphatiden als Grundlage für chromatographische Trennungen. Fette-Seifen-Anstrichmittel 78: 111 (1976).
12. LARSSON, K. The structure of mesomorphic phases and micelles in aqueous glyceride systems. Z. phys. Chemie (Neue Folge) 56: 173 (1967).
13. SHIPLEY, G. G., GREEN, J. P., and NICHOLS, B. W. The phase behavior of monogalactosyl, digalactosyl, and sulphquiovosyl diglycerides. Biochim. Biophys. Acta 311: 531 (1973).

14. FONTELL, K., EKWALL, P., MANDELL, L., and DANIELSSON, I. The structures of three mesomorphic phases in the sodium caprylate/decanol/water system. Acta Chem. Scand. 16: 2294 (1962).
15. LARSSON, K. On the structure of isotropic phases in lipid-water systems. Chem. Phys. Lipids 9: 181 (1972).
16. LARSSON, K., and LUNDSTROM, I. Liquid crystalline phases in biological model systems. In: Advances in Chemistry Series, Number 152, Lyotropic liquid crystals and the structure of biomembranes, ed. by S. Friberg, p. 43. Amer. Chem. Soc. (1976).
17. MORRISON, W. R., MANN, D. L., SOON, W., and COVENTRY, A. M. Selective extraction and quantitative analysis of non-starch and starch lipids from wheat flour. J. Sci. Fd. Agric. 26: 507 (1975).
18. TRAUB, W., HUTCHINSON, J. B., and DANIELS, D. G. H. X-Ray studies of the wheat protein complex. Nature 179: 769 (1957).
19. GROSSKREUTZ, J. C. A lipoprotein model of wheat gluten structure. Cereal Chem. 38: 336 (1961).
20. CHUNG, O. K., POMERANZ, Y., GOFORTH, D. R., SHOGREN, M. D., and FINNEY, K. F. Improved sucrose esters in breadmaking. Cereal Chem. 53: 615 (1976).
21. VAN LONKHUYSEN, H., and BLANKESTIJN, J. Influence of monoglycerides on the gelatinization and enzymatic breakdown of wheat and cassava starch. Stärke 28: 227 (1976).
22. DE STEFANIS, V. A., and PONTE, J. G. Studies on the breadmaking properties of wheat-flour nonpolar lipids. Cereal Chem. 53: 636 (1976).

[Received May 3, 1977. Accepted August 29, 1977]