Phase Equilibria in the Aqueous System of Wheat Gluten Lipids and in the Aqueous Salt System of Wheat Lipids

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ABSTRACT

The aqueous system of gluten lipids was studied for comparison with the wheat lipid-water system. Phase equilibria were determined and were illustrated in the form of a ternary system: nonpolar lipids/water/polar lipids. The structures of the phases were characterized by x-ray diffraction. Besides water and the nonpolar oil phase, three aqueous phases were observed. The lamellar phase and the reversed hexagonal liquid-crystalline phase were frequently observed in aqueous systems of polar lipids. A third liquid phase containing about 50–80% (w/w) water is analogous to the so-called L2-phase in simple amphiphile-water systems. The water system of wheat lipids is the first system of biological origin found to exhibit this phase. The functionality of the different phases with regard to baking is discussed on the basis of the phase properties. The physical behavior of extracted wheat lipids in the presence of a salt solution corresponding to that in a dough (0.260*M* NaCl, 0.070*M* KCl, 0.016*M* $MgCl_2$, and 0.004*M* $CaCl_2$) also was analyzed and related to phase equilibria and to corresponding structures in the aqueous system of wheat lipids. The degree of solubilization of nonpolar lipids in the lipid-water phases was substantially reduced when salt was present, and the phase equilibria involving the L2-phase was different.

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Phase equilibria and structures in the aqueous system of wheat lipids were recently reported (Carlson et al 1978). The phase behavior was described by a ternary phase diagram: water/polar lipids/nonpolar lipids. To obtain as much of the lipids as possible, they were extracted from wheat flour by water saturated n-butanol (WSB). To differentiate between the behavior of the total lipids obtained in this way and that of the lipids associated with the gluten fraction, we have similarly analyzed the aqueous system of lipids extracted from gluten.

The phase properties in lipid-water systems are well-known to be sensitive to the presence of electrolytes, particularly when the lipids contain ionic components (Larsson and Lundström 1976). Because a dough contains ions, the interaction between wheat lipids and salt solution is of interest. Within the lipids, the cations are of particular interest as counter-ions because all charged lipid species are anionic (Morrison et al 1975). As in our study of the wheat lipid aqueous system, WSB was used as solvent.

MATERIALS AND METHODS

The flour used was a Swedish spring wheat (AMY 1975), the same batch as studied earlier (Carlson et al 1978). Gluten was prepared with a semi-automatic gluten washer (Falling Number) and was used immediately. Wet gluten (67% [w/w] water) and 99% (w/w) ethanol were mixed to give a concentration of approximately 95% (w/w) ethanol in the gluten-solvent mixture. The mixture was homogenized in a Sorvall Omni-Mixer and stirred during extraction for 4 hr at 20°C. Because use of WSB in the gluten extraction resulted in formation of a stiff gel that was hard to handle in a reproducible way, ethanol was used. The extract was then centrifuged at 30,000 × *g* for 2 hr. The solvent was removed from the supernatant by a rotary vacuum evaporation at a temperature below 45°C. The collected lipid extract was dried over P_2O_5 for 24 hr before weighing and was stored at −18°C before use. The extraction was repeated twice. Aqueous samples were prepared immediately. Even after several weeks of storage, phase behavior did not differ. The flour was extracted as in the previous study (Carlson et al 1978).

Details concerning determination of the ratio of polar to nonpolar lipids, thin layer chromatography (TLC), preparation of lipid-water phases, and structure analysis of the different phases obtained were reported previously (Carlson et al 1978).

The salt solution (0.260*M* NaCl, 0.070*M* KCl, 0.016*M* $MgCl_2$, and 0.004*M* $CaCl_2$) was calculated to correspond to the salt concentration in a dough. The calculations are based on mineral amounts given for HRW-flour (Toepfer et al 1972) and on a concentration of 0.25*M* NaCl in the dough-liquor. The pH of the salt solution was approximately 5.

RESULTS AND DISCUSSION

Lipid Separation

The extractable lipid content of dry gluten was 6.8% (w/w). The lipid extract contained equal amounts of polar and nonpolar lipids. A TLC-pattern of the lipids extracted from flour and gluten is shown in Fig. 1.

Phase Behavior of Gluten Lipids-Water

The main features of the phase diagram for gluten lipids-water, illustrated in terms of three components (Fig. 2), are similar to those of the phase diagram of flour lipids-water (Carlson et al 1978). The principal phases were water, oil (nonpolar), and lipid-water phases. Two types of lipid-water phases were formed: a liquid phase with characteristics of an L2-phase and a liquid-crystalline phase. The liquid-crystalline phase consisted of a hexagonal liquid-crystalline phase of reversed type and a lamellar liquid-crystalline phase. The broken line in the phase diagram represents the binary system formed by gluten lipids and water.

Nonpolar lipids separated as a liquid oil phase on top of all sample compositions above 5% (w/w) water. At water contents up to about 15% (w/w), a reversed hexagonal liquid-crystalline phase was formed. At a water content of 15–40%, a lamellar liquid crystalline phase existed, which transformed into an L2-phase as the amount of water increased.

The significant differences between the phase behavior of gluten lipid-water systems (Fig. 2) and of wheat lipid-water systems (Fig. 3) were:

1) In the lamellar liquid-crystalline phase region of the gluten lipids-water system (Fig. 2), solubilization of nonpolar lipids increased as the water content increased, whereas in the same phase of the wheat lipid-water system the opposite was observed (Fig. 3).

2) The four-phase area, which deviated from the phase rule applied to the three-component system, was smaller in the gluten lipid-water system (Fig. 2) than in the wheat lipid-water system (Fig. 3). In the wheat system, this region disappeared as salt was included in the water, as described below.

3) The L2-phase existed up to 87% of water in the gluten lipid-water system (Fig. 2), whereas its maximum water content was about 78% in the wheat lipid-water system (Fig. 3). The two-phase region containing L2 and lamellar phases was also smaller in the gluten system. The L2-phase often contained small amounts of

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liquid-crystalline phase, however, and the border lines between these phases were therefore hard to establish.

Functionality of Lipids in Gluten

The existence of gluten depends on both its protein and its lipids. The state of association of the gluten lipids in gluten is not known. Different lipid-water phases have drastically different functional properties. What functional propertis of gluten can be attributed to the lipids? The water distribution between the components in a dough is not known, and therefore the lipid phase that is formed is not known. If we consider the different possible phases, however, the lamellar liquid-crystalline phase would be expected to contribute to baking properties. The most important property of this phase is its surface activity; it can form either hydrophobic or hydrophilic interfaces according to the environment. The lamellar liquid-crystalline phase is, therefore, very efficient as a dispersing and lubricating agent, in contrast to the hexagonal liquid-crystalline phase and the L2-phase, which in these respects behave like a plastic fat and a nonpolar oil, respectively.

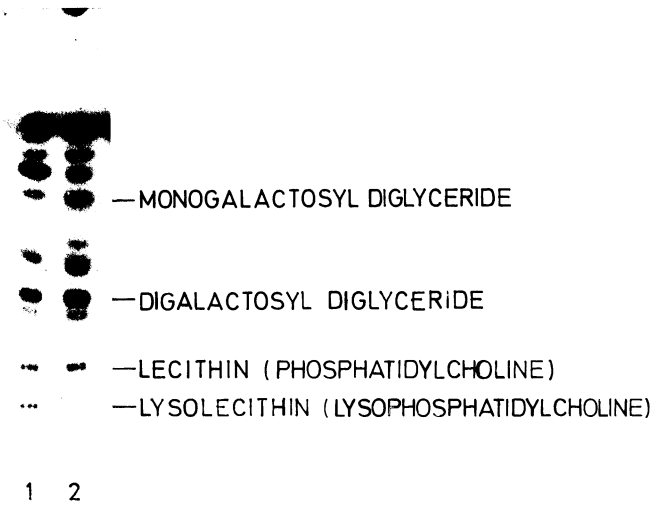

Fig. 1. TLC-pattern of wheat lipids. 1, Water saturated *n*-butanol extract from AMY flour. **2,** 95% ethanol extract from AMY-gluten. The solvent system used chloroform-methanol-water (65:25:4); 200 μg lipids were applied from each extract.

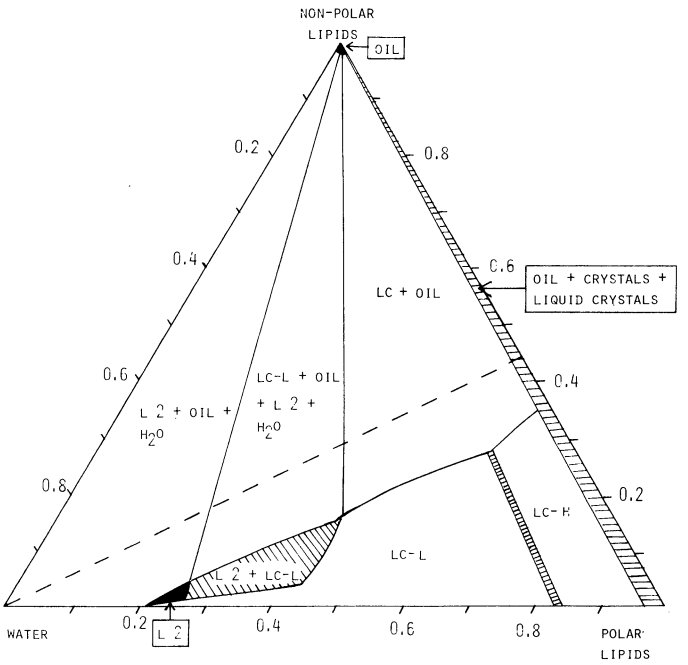

Fig. 3. Phase diagram of wheat lipids and water at 25° C from previous study (Carlson et al 1978). **LC** = liquid-crystalline phase. **LC-L** = lamellar liquid-crystalline phase. **LC-H** = reversed hexagonal liquid-crystalline phase. The broken line corresponds to the binary system of the total extracted lipids and water.

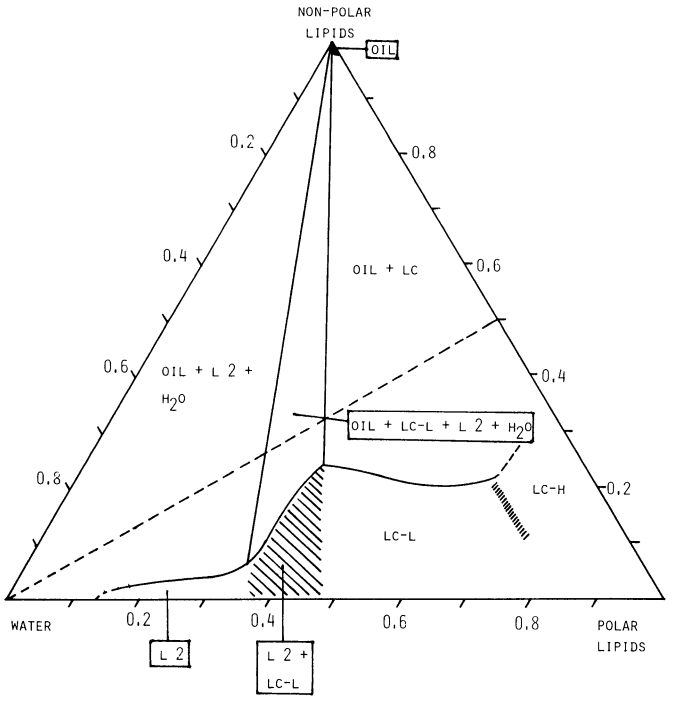

Fig. 2. Ternary phase diagram of gluten lipids at 25° C represented by water, polar, and nonpolar lipids. **LC** = liquid-crystalline phase. **LC-L** = lamellar liquid-crystalline phase. **LC-H** = reversed hexagonal liquid-crystalline phase. The broken line corresponds to the binary system of the total extracted lipids and water.

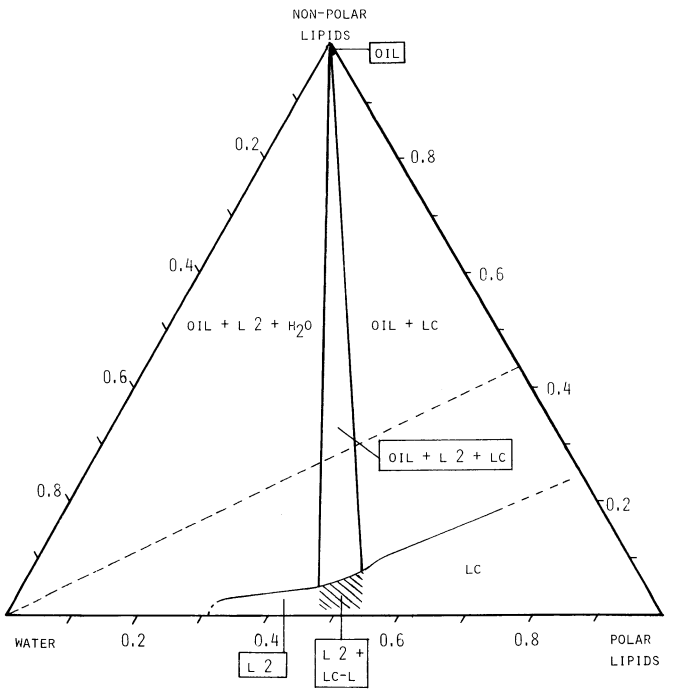

Fig. 4. Ternary phase diagram of wheat lipids and an aqueous salt solution at 25° C. **L** = liquid-crystalline phase. **LC-L** = lamellar liquid-crystalline phase. **LC-H** = reversed hexagonal liquid-crystalline phase. The broken line corresponds to the binary system of the total extracted lipids and water.

Wheat Lipids and Aqueous Salt Solution

As defined by a ternary system, the phase behavior of the aqueous salt solution and the extracted wheat lipids, divided into polar and nonpolar components, is given in Fig. 4. When this phase diagram is compared with that of the aqueous system (Fig. 3), the most striking effect of the presence of ions is the reduction of the degree of solubilization of nonpolar lipids in the liquid-crystalline phase and in the L2-phase. The lamellar liquid-crystalline phase exhibited a one-dimensional swelling to a maximum lamellar thickness of 73 Å at a water content of about 40% (w/w). The value of the lipid bilayer thickness of this phase, obtained by extrapolation to a water content of zero, is 42 Å, which is much smaller than the corresponding bilayer of the saltfree system (Carlson et al 1978). The salt-containing system apparently accommodates the triglyceride molecules without swelling the bilayer, which must mean that the triglyceride chains are mainly parallel to the chains of the polar lipids. In the saltfree system, the triglyceride molecules can enter the gap between the hydrocarbon chain tails of the bilayer and therefore increase its thickness.

The amount of hexagonal liquid-crystalline phase was very small at all water compositions. By ultracentrifugation, this phase separated as a small precipitate at the bottom of the tube, together with contaminating colloidal aggregates of protein and starch granules. We believe that this phase is related to the presence of divalent ions; the presence of traces of divalent ions in an aqueous system of negatively charged lipids favors the formation of the reversed hexagonal phase (Rand and Sen Gupta 1972). A few lipid-water mixtures with 0.26*M* sodium chloride in the water also were examined, and it was evident that the relative amounts of the L2-phase and the liquid-crystalline phase changed to some extent, indicating that the small number of divalent ions have a significant effect on the phase equilibria.

ACKNOWLEDGMENT

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