Enzymatic Hydrolysis of Oat and Soya Lecithin: Effects on Functional Properties

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Enzymatic hydrolysis of oat and soy lecithins and its effects on the functional properties of lecithins were investigated. The phospholipase used was most efficient at low enzyme and substrate concentrations. More fatty acids were released from soy lecithin than from oat lecithin. The maximum degree of hydrolysis was 760 μmol free fatty acids per gram soy lecithin and 170 μmol free fatty acids per gram oat lecithin. On the basis of the total carbohydrate and phosphorus contents in the polar fractions of the lecithins, oat lecithin contained more glycolipids and less phospholipids than soy lecithin. With regard to functional properties, the stability of oil-in-water emulsions was enhanced by hydrolyzed soy lecithin and by crude and hydrolyzed oat lecithins, but only hydrolyzed soy lecithin prevented the recrystallization of barley starch. The dissociation enthalpy of amylose–lipid-complex (AML-complex) was significantly higher when hydrolyzed soy lecithin was present. Hydrolyzed oat lecithin slightly affected the dissociation enthalpy of AML-complex. The other lecithins had no effect on recrystallization or dissociation enthalpies in the barley–starch matrix.

KEY WORDS: DSC, emulsion stability, hydrolysis, lecithin, lipid-starch-interactions, phospholipase.

The annual world production of lecithins from different plant sources is 145,000 tons, 90% of which is soy lecithin. Soy lecithin is especially useful in the food industry (1). The emulsifying properties of natural lecithin need improvement because the emulsifying activities of the phospholipid components compete with each other (2). Better emulsifiers are obtained from lecithin by physical, chemical or enzymatic modifications.

Fractionation of crude lecithin by 90% ethanol yields a good oil-in-water emulsifier, which is rich in phosphatidylcholine (PC). The sensitivity to calcium ions is diminished by the removal of phosphatidylethanolamine (PE). The fraction containing PE is a good water-in-oil emulsifier (3). Together, PC and PE form liposomes that prevent bread from getting stale (2).

Chemical modification of lecithin includes nonspecific hydrolysis, acetylation and hydroxylation. Chemical hydrolysis produces a dark-colored, more hydrophilic lecithin. The double-ionic properties of PE are blocked in acetylated lecithin, thus the oil-in-water emulsifying properties are enhanced. More hydrophilic lecithins are also achieved by hydroxylation of unsaturated fatty acids and of the amino group of PE. Hydroxylated products are easily dispersed in water (3).

Most of the few reports concerning enzymatic hydrolysis or modification of lecithins have appeared in Japanese scientific literature (4–7), with some exceptions (8–11). According to Erdelyi (11), enzymatic hydrolysis of lecithin by phospholipase A_2 yields lysolecithins, which have a pronounced effect on oil-in-water emulsions. Addition of lysolecithins to nonionic food surfactants improves the stability of oil-in-water emulsions (12). Emulsifiers that are more hydrophilic than those used at present are needed in the margarine industry, especially in low-calorie spreads and low-salt products for improvement of aroma release, as well as for their traditional effects, such as antispattering and the prevention of sandiness (13). Lysolecithin satisfies these requirements in a natural way.

Phospholipids and glycolipids have similarities in their structure, having both hydrophilic and lipophilic groups in the molecule (14). Thus, it is probable that they can also act as stabilizers in oil-in-water emulsions and as emulsifiers in starch gels. Lysophospholipids form amylose–lipid-complexes (AML-complex), which lower the gelatinization enthalpy of starch (15), and probably also bind to the amylopectin molecule, thus retarding recrystallization of the starch (16). Gelatinization and the presence of AML-complex can be analyzed with a differential scanning calorimeter (DSC) (15,16).

In the present work, the conditions of the enzymatic hydrolysis of oat and soy lecithins were studied in relation to their functional properties. The emulsion stability of oil-in-water emulsions was studied, and interactions of barley starch with different lecithins as emulsifiers were investigated.

EXPERIMENTAL PROCEDURES

Materials. Oat oil was extracted from milled oat bran with 92% ethanol at 78°C. The extract was centrifuged and filtered, and then the solvent was evaporated. Neutral lipids were removed from the oil by supercritical carbon dioxide extraction (350 bar, 50°C) (17). The polar fraction was called oat lecithin. Soy lecithin was kindly donated by Mildola Oy (Kirkkonummi, Finland) and was deoiled by extraction of neutral lipids with acetone. Reference emulsifiers were as follows: MC THIN AF-1 (standard soy lecithin; Lucas Meyer, Hamburg, Germany), Emulfluid E (enzymatically hydrolyzed soy lecithin; Lucas Meyer) and Emulfluid A (acetylated soy lecithin; Lucas Meyer); Dimodan LS (distilled monoglyceride from sunflower oil; Grindstedt Products, Braband, Denmark) and Triodan 20 (polyglycerol ester made from edible oil; Grindstedt Products). All reagents were of analytical grade. Lecitase (phospholipase A_2) was purchased from Novo Nordisk (Bagsveard, Denmark).

Hydrolysis of lecithins. Lecithin was hydrolyzed in calcium chloride solution (5 mM) or in deionized water at an initial pH of 9.0. Hydrolysis was started by the addition of Lecitase solution. The degree of hydrolysis was analyzed immediately after incubation by titrating (0.1M NaOH) the samples to an endpoint of pH 9.5. When the effects of the reaction conditions were studied, the substrate concentration was 2%. Lecitase was added at 80 nkat/g lecithin, and the incubation was performed at 60°C with magnetic stirring (300 rpm) and terminated by titration after 0, 2, 4, 6 and 24 h, unless otherwise stated.

Total carbohydrate content. The polar fractions of oat and soy lecithins were saponified by addition of 1 mL of 0.5N sodium hydroxide solution in methanol, by incubation for 1.5 h at 60°C and by addition of 1.5 mL of 6N

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hydrochloric acid. Saponified fatty acids were extracted three times with *n*-heptane (2 mL), and the methanol-water phase was used for the carbohydrate analysis. The total carbohydrate content was analyzed by the method of Dubois *et al.* (18).

Total phosphorus content. The total phosphorus content of oat and soy lecithins was determined by the American Oil Chemists' Society molybdenum blue method (19) after dry ashing.

Emulsion test. The emulsifier was dissolved either in rapeseed oil or in water at 0.1% (wt/vol) of the total emulsion volume. Two oil-in-water emulsions for each emulsifier were made from rapeseed oil and water (2:3, vol/vol) by rapid homogenization with an Ultra-Thurrax homogenizer for 60 s at 50°C. The emulsion was poured into four scaled test tubes. Emulsion stability was expressed as the time (min) required for clear separation of the water phase at room temperature when visually assessed against light.

DSC analysis. The effects of the lecithins on the thermal behavior of barley starch were studied in a DSC (Mettler DSC 30S, Greitensce, Switzerland). The thermograms of gelatinization and dissociation of the AML-complex were measured in medium pressure pans (Mettler ME-26929), and the thermograms concerning recrystallization were measured in a standard aluminum pan (Mettler ME-27331). Moisture content was 50%, and sample size was about 5 mg of starch. Thermograms were recorded in triplicate from 10 to 100°C or 150°C at a scan rate of 10°C/min. Re-scans were performed for studying starch recrystallization after storage of the gelatinized samples at 4°C for different periods of time.

RESULTS AND DISCUSSION

Enzymatic hydrolysis of lecithin. Calcium ions increased the rate and the extent of hydrolysis (Fig. 1). As compared with distilled water, the initial rate of hydrolysis of oat lecithin was three times faster when calcium ions were present. Erdelyi (11) studied enzymatic hydrolysis of sunflower lecithin and reported that Lecitase hydrolyzed lecithin both in the presence and in the absence of calcium ions. This is in accordance with our results, but we observed a significant increase in the hydrolysis when calcium ions were present.

Soy lecithin was hydrolyzed to a greater extent than oat lecithin, as measured by the amount of fatty acids released (Fig. 2). The maximum degree of lecithin hydrolysis was 760 μmol free fatty acids per gram soy lecithin and 170 μmol free fatty acids per gram oat lecithin in 2% lecithin solutions. The total contents of phosphorus and carbohydrate of oat and soy lecithins indicated that soy lecithin contains more phospholipids than oat lecithin and that the amount of glycolipids in oat lecithin is significantly higher than that in soy lecithin (Table 1). This could also mean that only phospholipids are hydrolyzed and that the glycolipids stay intact in the reaction. The lysophospholipid content of unhydrolyzed lecithins was not determined. It is also possible that oat and soy lecithins originally contained different amounts of lysophospholipids. After achieving equilibrium (6 h), the amount of released fatty acids from oat lecithin remained constant, even after an extended period of time (72 h, results not shown). The enzyme was more efficient when the lecithin

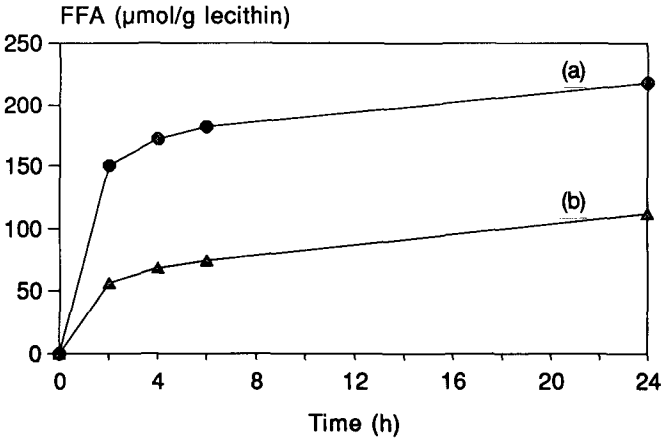

FIG. 1. Hydrolysis of 2% oat lecithin solution at 60°C: (a) in 5 mM $CaCl_2$ solution; (b) in distilled water. Lecitase (Novo Nordisk, Bagsveard, Denmark) at a dose of 80 nkat/g lecithin was used, and the reaction mixtures were stirred magnetically (300 rpm). FFA, free fatty acids.

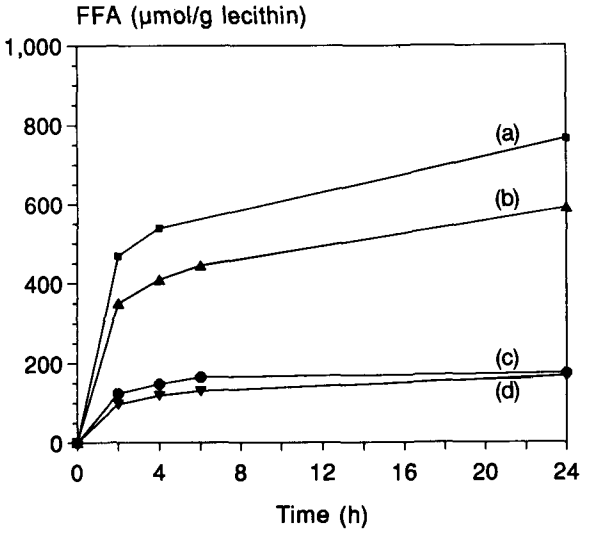

FIG. 2. Effect of substrate concentration on the hydrolysis of oat and soy lecithins at 60°C with Lecitase (80 nkat/g lecithin, stirring rate 300 rpm) in 5 mM $CaCl_2$ solution: (a) 2% soy lecithin; (b) 5% soy lecithin; (c) 2% oat lecithin; and (d) 5% oat lecithin. Source and abbreviation as in Figure 1.

TABLE 1

The Total Phosphorus and Carbohydrate Contents of the Polar Fractions of Soy and Oat Lecithins

Lecithin	Phosphorus content (%)	Carbohydrate content (%)
Soy lecithin (acetone-insoluble)	3.0	10
Oat lecithin (supercritical carbon dioxide)	1.4	22

was at low concentrations, because the solutions were less viscous.

The maximum beneficial amount of enzyme used was 80,000 nkat/g lecithin (corresponding to the logarithmic value of 4.90 in Fig. 3a). Further additions did not increase the release of fatty acids. The degree of hydrolysis of oat lecithin increased concomitantly with the enzyme concentration (Fig. 3b), but the increase was not efficient. When the enzyme concentration was increased from 80 to 8,000 nkat/g lecithin, the amount of free fatty acids liberated from a 2% solution of oat lecithin increased only by 70 μmol (from 150 to 220 μmol) per gram of lecithin. The lower enzyme concentrations could not be compensated for by extended incubation time (Fig. 3b).

The maximum degree of hydrolysis of lecithin in 24 h was achieved at 60–70°C (result not shown), which was in agreement with the information provided by Novo Nordisk concerning the effect of temperature on the enzymatic hydrolysis of egg yolk (0.4% phospholipids) catalyzed by Lecitase. Thus, the optimum temperature of hydrolysis is not dependent on the origin of the lecithin.

Hydrolysis of oat lecithin can be controlled to a certain extent by temperature, viscosity of the substrate, time and enzyme concentration. These results are in accordance with those of Erdelyi (11). The reason for the low degree of hydrolysis may have been the end-product inhibition or the inhibition by the low pH resulting from the release of fatty acids.

Functional properties of the modified lecithins. The enzymatically hydrolyzed soy lecithins (Bars 2 and 6) were the best stabilizers in this study (Fig. 4). The difference in stability between the two hydrolyzed soy lecithins was probably due to the greater degree of hydrolysis in the sample prepared in this study (Bar 2). The stability of the emulsions containing oat lecithins was independent of the degree of hydrolysis of the lecithin (Bars 3 and 4), but the stabilizing effect of the hydrolyzed soy lecithin increased concomitantly with the degree of hydrolysis (Fig. 5). The different unhydrolyzed soy lecithins (Bars 1 and 5), as well as monoglyceride (Bar 8), formed equally stable emulsions with rapeseed oil and deionized water (Fig. 4). Monoglycerides and polyglycerol esters (Bars 8 and 9) are normally used in margarines in water-in-oil emulsions and are more lipophilic. This explains their relatively poor performance in the oil-in-water emulsion test. The difference between values obtained for the deoiled soy lecithin in Figures 4 and 5 was due to variations between batches of lecithin.

The emulsions containing oat lecithin were almost as stable as the emulsions stabilized by hydrolyzed soy lecithin. Free fatty acids released in the hydrolysis were present in the emulsions. Thus, the stabilization of the emulsions could be the combined effect of both hydrolysis products, lysophospholipids and free fatty acids. The free fatty acids are probably less important in stabilizing the

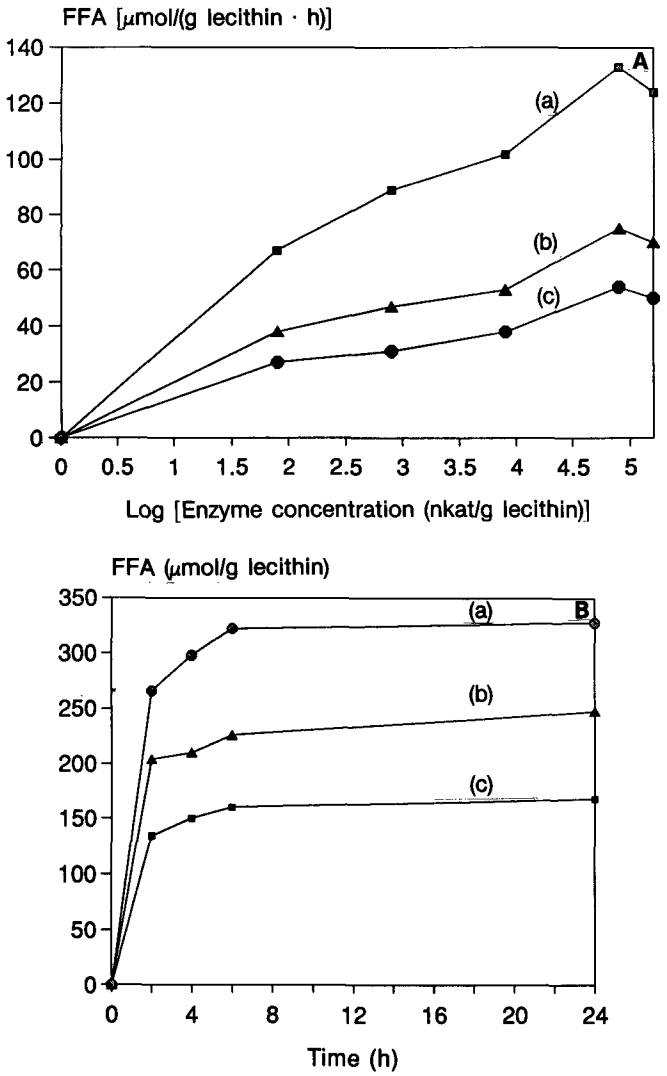

FIG. 3. A. Effect of enzyme concentration on the rate of hydrolysis in 2% oat lecithin. The logarithmic scale corresponds to enzyme activities from 80 to 167,000 nkat/g lecithin. Hydrolysis times were: (a) 2 h; (b) 4 h and (c) 6 h. B. Effect of enzyme concentration on the hydrolysis of 2% oat lecithin: (a) 80,000 nkat/g lecithin; (b) 8,000 nkat/g lecithin; and (c) 80 nkat/g lecithin. Other hydrolysis conditions and abbreviation as in Figure 2.

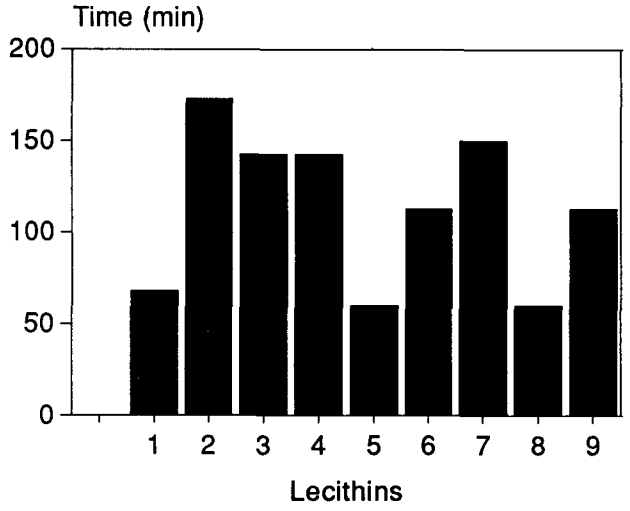

FIG. 4. Effect of emulsifier (0.1%) on the emulsion stability of rapeseed oil-water emulsion (2:3, vol/vol). Emulsifiers: 1, Deoiled soy lecithin; 2, hydrolyzed soy lecithin; 3, deoiled oat lecithin; 4, hydrolyzed oat lecithin; 5, MC THIN AF-1 (standard soy lecithin; Lucas Meyer, Germany); 6, emulfluid E (enzymatically hydrolyzed soy lecithin; Lucas Meyer); 7, emulfluid A (acetylated soy lecithin; Lucas Meyer); 8, dimodan LS (monoglyceride, Grindstedt Products, Braband, Denmark); 9, triodan 20 (polyglycerol ester; Grindstedt Products).

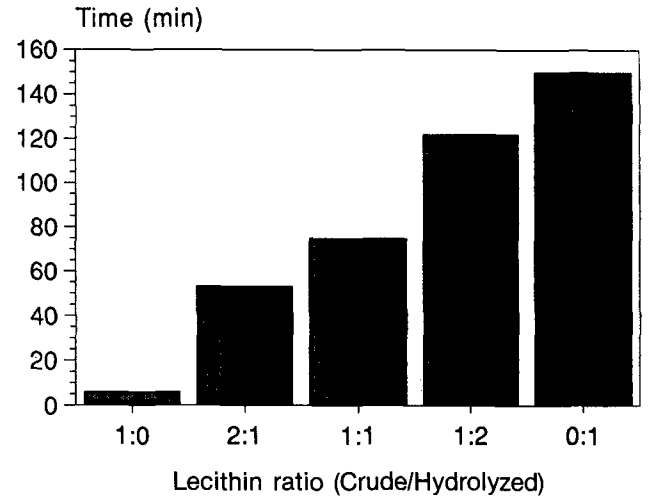

FIG. 5. Effect of the degree of hydrolysis of soy lecithin on the emulsion stability of rapeseed oil/water emulsions. Hydrolysis was performed at 60°C in 5 mM $CaCl_2$ for 24 h. Crude (deoiled) and hydrolyzed lecithins were mixed in the ratios 1:0, 2:1, 1:1, 1:2 and 0:1 (w/w).

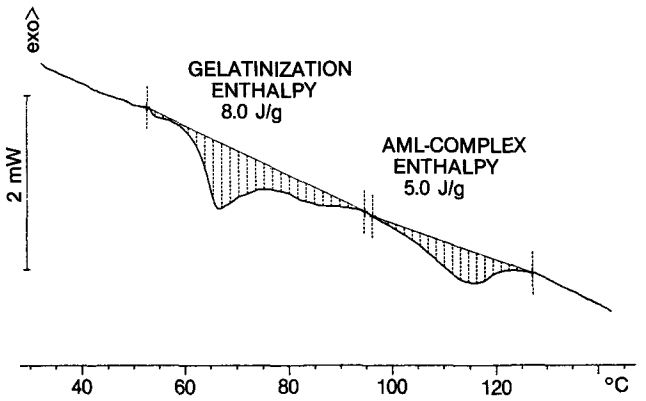

FIG. 6. The endotherms of gelatinization and dissociation of amylase-lipid-complex (AML-complex) in the barley-starch matrix containing 3.5% of hydrolyzed soy lecithin at 50% moisture.

TABLE 2

The Effect of Added Lecithins (3.5%) on the Gelatinization Enthalpy [ΔH(gel)] and Dissociation Enthalpy [ΔH(complex)] of Amylose-Lipid-Complex in Barley Starch[a]

Source of lecithin	ΔH(gel) (J/g dry matter)	ΔH(complex) (J/g dry matter)
No addition	11	1.2
Soy	10	0.8
Hydrolyzed soy	8.0	5.0
Oat	10	1.1
Hydrolyzed oat	9.3	2.3

[a]As analyzed by differential scanning calorimetry at 50% moisture.

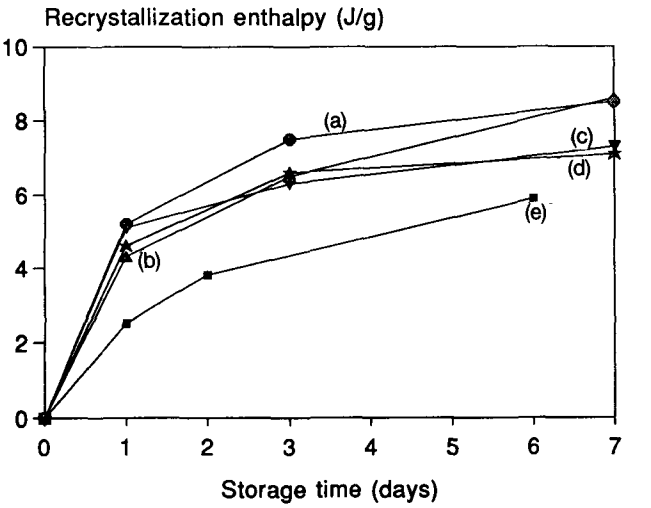

FIG. 7. Effect of lecithins on the recrystallization of barley starch; 50% moisture content. Lecithins (3.5%): (a) No addition; (b) soy lecithin; (c) oat lecithin; (d) hydrolyzed oat lecithin; and (e) hydrolyzed soy lecithin.

oil-in-water emulsions because the unhydrolyzed oat lecithin stabilized the emulsion just as well. Because the polar fraction of oat lecithin contained twice as much carbohydrates as the polar fraction of soy lecithin (Table 1), glycolipids could probably act as stabilizers in oil-in-water emulsions.

All the lecithins studied decreased the gelatinization enthalpy of barley starch, but only hydrolyzed lecithins showed higher enthalpy of the AML-complex as compared with the sample without lecithin addition (Table 2 and Fig. 6). Hydrolyzed oat lecithin had a small effect on the dissociation enthalpy of AML-complex. The effect of hydrolyzed soy lecithin was much higher than that of hydrolyzed oat lecithin, probably because of its higher lysolecithin content. It is well known that lysophospholipids and fatty acids are able to complex with amylose (20,21). Because the fatty acids were not removed from the hydrolyzed lecithins, it is not possible to decide whether lysophospholipids were the only interacting lipids in this study. Despite the fact that hydrolyzed soy lecithin is a complex mixture of phospholipids, lysophospholipids, fatty acids and glycolipids, the result was in accordance with the earlier study (15) concerning the effect of lysolecithin on wheat starch gelatinization and AML-complex. Unhydrolyzed soy and oat lecithins were incapable of complexing with amylose of barley starch due to their low content of lysophospholipids and fatty acids.

Hydrolyzed soy lecithin clearly retarded recrystallization of barley starch, but the effect of the other lecithins was insignificant (Fig. 7). Glycolipid- rich oat lecithins did not influence the recrystallization of barley starch, but the recrystallization was retarded from one day for starch without lecithin to six days by using 3.5% hydrolyzed soy lecithin.

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