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Alterations of Soybean Lecithin during Curd Formation in Cheese Making

Nikolaus Weber,* Arnold Wiechen, Wolfgang Buchheim, and Dieter Prokopek

The alterations of soybean lecithin during curd formation in cheese making have been studied with radioactive phosphatidylcholines and phosphatidylethanolamines. It was found that most of the added mixture of radioactive glycerophospholipids was not changed when processing the milk. Minor proportions of the labeled phospholipids were metabolized by starter and *Penicillium* cultures. Radioactive lipophilic hydrolysis products and radioactive hydrophilic metabolites of microbial origin both derived from labeled glycerophospholipids that had been added to cheese milk were concentrated predominantly in whey.

It has been reported that in making cheese the yield of curd can be increased by adding phosphatidylcholines (lecithins) to the milk (Bily, 1981). The characteristic feature of this process is that lecithin is added at a level of 0.001–0.15% to milk prior to the precipitation of the curd. The lecithin may be added to cheese milk at any period of time before the point of coagulation is reached.

We have recently described the distribution of added soybean phospholipids between curd and whey as well as the formation of casein containing lecithin liposomes that can be built into the rennet jelly when making cheese (Wiechen et al., 1985). In the present paper we report the turnover of phosphatidylcholines and phosphatidylethanolamines in curd and whey during the first steps of Camembert cheese making. The conversion of added soybean phospholipids is of interest, not only from a technological point of view but also with regard to food legislation.

EXPERIMENTAL SECTION

Chemicals. [1-^{14}C]Linoleic acid (specific activity 2.03 GBq/mmol) was purchased from Amersham Buchler (D-3300 Braunschweig, FRG). Commercial soybean lecithin, Metarin K, containing 20–23% of phosphatidylcholines and 21–24% of phosphatidylethanolamines was a product of Lucas Meyer GmbH, D-2000 Hamburg, FRG.

Determination of Radioactivity. Solutions were mixed with Aquasol-2 (NEN-Chemicals, D-6072-Dreieich, FRG), and radio activity was determined by liquid scintillation counting in a Tri-Carb C 2425 instrument (Packard Instruments Co., Downers Grove, IL 60515). The distribution of radioactive fractions of thin-layer chromatograms was determined with a Berthold TLC scanner, Model LB 2760, or with a Berthold Automatic TLC linear analyzer, Model LB 2832, in combination with a data acquisition system LB 500 (BF-Vertriebsgesellschaft, D-7547 Wildbad, FRG). After two-dimensional TLC, labeled fractions were detected with the automatic TLC linear analyzer system and evaluated by using a perspective TLC program. Radio gas chromatography was carried out in a Perkin-Elmer F 22 instrument (Perkin-Elmer Bodenseewerk, D-7770 Überlingen, FRG) equipped with thermal conductivity detectors. Methyl esters of fatty acids were analyzed on a glass column, 1.8 m × 4 mm, packed with 10% Silar 5 CP on Gas Chrom Q, 100–120 mesh (Applied Science Laboratories, State College, PA 16801) at 180 °C with helium as the carrier gas at a flow rate of 40 mL/min. Radioactivity in the carrier gas effluent was monitored in a Packard gas proportional counter, Model 894 (Packard Instruments Co.), combined with a Spectra Physics SP 4270 integrator (Spectra-Physics, D-6100 Darmstadt, FRG).

Cell Suspension Cultures of Soya. Heterotrophic cell suspension cultures of soya were propagated in B_5 medium (Gamborg et al., 1968) containing 2×10^{-6} M (2,4-dichlorophenoxy)acetic acid. The soya cells were shaken in the dark at 25 °C and subcultured every 10–14 days.

Preparation of Radioactively Labeled Phosphatidylcholines and Phosphatidylethanolamines. Heterotrophic soya cells, 10 g of cells/30 mL of preconditioned medium, were preincubated for 1 h under anaerobic conditions (argon atmosphere) and then added to 370 kBq (1.0 μmol) of [1-^{14}C]linoleic acid in 0.02 mL of 80% aqueous ethanol–diethyl ether (1:2, v/v). The cells were incubated at 25 °C for 0.5 h under anaerobic conditions. The cells were collected by repeated centrifugation and washing with 0.1 M sodium phosphate–potassium phosphate buffer of pH 6.0. The pellets were suspended in 2 mL of 2-propanol and heated in closed tubes at 100 °C for 10 min. The cells were homogenized, and the lipids were extracted according

Bundesanstalt für Fettforschung, Institut für Biochemie und Technologie, H. P. Kaufmann-Institut, D-4400 Münster, Federal Republic of Germany (N.W.), and Bundesanstalt für Milchforschung, Institut für Chemie und Physik (A.W. and W.B.), and Institut für Verfahrenstechnik (D.P.), D-2300 Kiel, Federal Republic of Germany.

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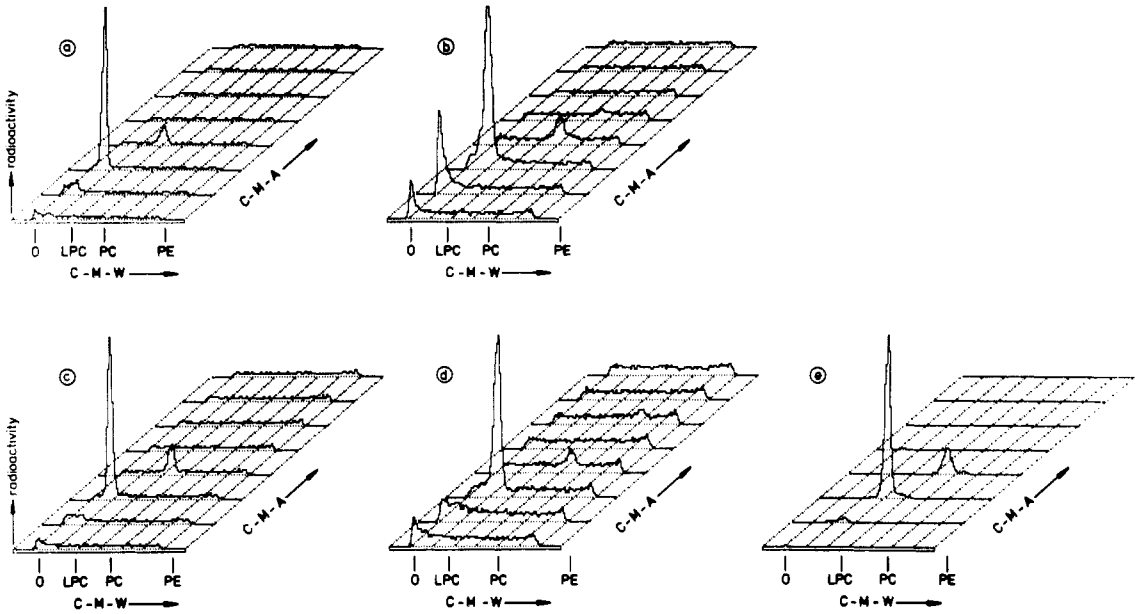

Figure 1. Two-dimensional thin-layer chromatography of radioactive total polar lipids of curd and whey processed under various conditions as well as of the mixture of radioactive phosphatidylcholines and phosphatidylethanolamines isolated from soybean cells that had been added to cheese milk: (a) experiment 1 curd; (b) experiment 1 whey; (c) experiment 2 curd; (d) experiment 2 whey; (e) soya cells. (The distribution of radioactivity on chromatoplates was determined with the Berthold linear analyzer system and presented on a Perspective TLC program, as described in the Experimental Section. Two-dimensional thin-layer chromatograms were developed in chloroform–methanol–water (65:25:4, v/v) [C–M–W, first direction] and chloroform–methanol–concentrated ammonia (65:25:5, v/v) [C–M–A, second direction]. Key: O = Origin; PC = phosphatidylcholines; LPC = lysophosphatidylcholines; PE = phosphatidylethanolamines.

Table I. Distribution of Radioactivity in Total Lipids and Various Lipid Fractions Isolated from Curd and Whey

expt[a]	fraction	total lipids, mg/g dry wt	radioact in total lipid extract, %	distribn of radioact, %					
				lyso-phosphatidyl-cholines	phosphatidyl-cholines	phosphatidyl-ethanol-amines	other polar lipids[b]	diacyl-glycerols	fatty acids
1	curd	13	>97	4	62	14	7	6	7
	whey	14	90	7	46	10	23	9	5
2	curd	15	>97	3	69	16	5	4	3
	whey	15	92	3	57	10	13	11	6
soya cells				2	77	15	6	0	0

[a] Experimental conditions as described in the Experimental Section. [b] Including origin, lysophosphatidylethanolamines, and phosphatidic acids.

Table II. Stereospecific Distribution of Radioactivity in Acyl Moieties between the *sn*-1 and *sn*-2 Positions of Phosphatidylcholines and Phosphatidylethanolamines from Curd and Whey as well as from Soya Cells

expt	fraction	glycero-phospholipids[a]	stereosp distribn of radioact acyl moieties, %		compn of radioact acyl moieties,[b] %: [^{14}C]linoleoyl
			sn-1	*sn*-2	
1	curd	PC	36	64	>95
		PE	34	66	
	whey	PC	37	63	>95
		PE	22	78	
2	curd	PC	36	64	>95
		PE	34	66	
	whey	PC	27	73	>95
		PE	39	61	
soya cells		PC	38	62	>97
		PE	37	63	

[a] Phosphatidylcholines (PC), phosphatidylethanolamines (PE). [b] In mixtures of phosphatidylcholines plus phosphatidylethanolamines.

to an established procedure (Bligh and Dyer, 1959).

Isolation and Analysis of Radioactively Labeled Lipids of Soya Cells. The total lipids extracted from the incubation mixture were fractionated by TLC on silica gel with chloroform–methanol–water (65:25:4, v/v) (Wagner et al., 1961), and the distribution of radioactivity in the various fractions was determined by scanning. The fractions of phosphatidylcholines (R_f 0.25) and phosphatidylethanolamines (R_f 0.48) were isolated.

The two fractions of phosphatidylcholines and phosphatidylethanolamines were purified by repeated TLC with chloroform–methanol–water (65:25:4, v/v). After elution from the adsorbent with chloroform–methanol–water (5:10:4, v/v), their purity was checked by two-dimensional TLC on silica gel with chloroform–methanol–water (65:25:4, v/v) in the first direction and chloroform–methanol–concentrated ammonia (65:25:5, v/v) (Rouser et al., 1970) in the second. The radiopurity of the two

combined fractions was checked with the automatic TLC analyzer system (Figure 1e). The yield of the combined fractions of radioactive phosphatidylcholines and phosphatidylethanolamines was about 80% of the added [1-^{14}C]linoleic acid; the specific activity was 7.4 MBq/g of phospholipids. Analytical data are given in Tables I and II.

An aliquot of the mixture of radioactive glycerophospholipids was transmethylated; the resulting mixture of methyl esters of fatty acids was purified by TLC and analyzed by GLC (Chalvardjian, 1964).

Experimental Conditions of Curd Formation. The conversion of labeled phosphatidylcholines and phosphatidylethanolamines was studied under various experimental conditions as described recently (Wiechen et al., 1985). Commercial soya lecithin, 1 g in 10 mL of petroleum hydrocarbon, was labeled by adding 2.8 μCi of a mixture of ^{14}C-labeled phospholipids that were prepared as described above. After evaporation of the solvent the labeled lecithin was dispersed by sonification in 30 mL of skim milk at 70 °C.

Distribution and metabolism of radioactive lecithin were studied under various experimental conditions:

Experiment 1. Pasteurized cheese milk was mixed with a nonprocessed dispersion of labeled lecithin and acidified separately by a culture that was prepared by growing 0.8 mL of starter culture in 30 mL of sterile skim milk for 20 h at 22 °C.

Experiment 2. The dispersion of labeled lecithin in 30 mL of skim milk was sterilized (130 °C, 15 min) and then inoculated with 0.8 mL of starter culture. After the culture was grown for 20 h at 22 °C, this acidified dispersion was added to cheese milk.

In both experiments, cheese was made under identical conditions: Dispersions of lecithin in skim milk containing starter cultures were made with pasteurized cheese milk (fat content 3%), containing 0.02% of a culture of *Penicillium candidum* and 1 mL of 20% $CaCl_2$ solution, to a total amount of 2 kg. After preincubation for 1 h at 32 °C, rennet, 0.4 mL (rennet strength 1:10.000), was added to the cheese milk and incubation was continued for 1 h at 32 °C. Finally, curd and whey were separated, freeze-dried, and analyzed for radioactive metabolites of lecithin.

Extraction of Lipids from Curd and Whey. Curd and whey from both experiments were freeze-dried. The lipids of these samples were extracted with chloroform–methanol–water (5:10:4, v/v) according to an established procedure (Folch et al., 1957) and dried.

Analysis of Radioactive Lipids of Curd and Whey. Total lipids of curd and whey were fractionated by TLC on silica gel as described above. The fractions of neutral lipids (R_f 0.8–1.0), phosphatidylcholines, and phosphatidylethanolamines were each isolated.

The fraction of neutral lipids was resolved by TLC on silica gel with hexane–diethyl ether–acetic acid (80:20:1, v/v) twice, and the radioactivity in the fractions of unesterified fatty acids (R_f 0.65) and diacylglycerols (R_f 0.15–0.25) was determined with the scanner. The fractions of unesterified fatty acids were methylated by diazomethane. The resulting methyl esters of fatty acids were identified by cochromatography with a standard on silica gel using hexane–diethyl ether (80:20, v/v) as the solvent system. The fractions of diacylglycerols were identified by cochromatography with a standard on silica gel using the same solvent system.

Fractions of phosphatidylcholines and phosphatidylethanolamines were purified by rechromatography on silica gel with chloroform–methanol–concentrated ammonia (65:25:5, v/v) and identified by cochromatography with standards. Both fractions showed a molybdenum blue color with Dittmer and Lester (1964) reagent. Finally, phosphatidylcholines were identified by giving a positive reaction with Dragendorff reagent (Wagner et al., 1961), whereas phosphatidylethanolamines gave a positive reaction with ninhydrin (Skipski et al., 1962).

Stereospecific Analysis of Acyl Moieties at the *sn*-1 and *sn*-2 Positions of Diacylglycerophosphocholines and Diacylglycerophosphoethanolamines. About 2 mg of the two glycerophospholipids from curd and whey were hydrolyzed with phospholipase A_2 from hog pancreas (Boehringer Mannheim, D-6800 Mannheim, FRG) and analyzed as described earlier (Weber and Benning, 1985).

RESULTS AND DISCUSSION

Radioactively labeled phosphatidylcholines and phosphatidylethanolamines were isolated from soybean cells in suspension culture that had been incubated with [1-^{14}C]linoleic acid (Stumpf and Weber, 1977). The mixture of radioactive glycerophospholipids from soya cells showed a close resemblance to the commercial soya lecithin, which was used to increase the yield of cheese. In studies concerned with the distribution (Wiechen et al., 1985) or alteration of lecithin it is obviously of advantage to use a mixture of these phospholipids rather than the individual molecular species of these classes of compounds.

Freeze-dried aliquots of curd and whey from both experiments contained similar proportions of total lipids that consisted mainly of triacylglycerols. The radioactive compounds of curd were almost completely extracted with chloroform–methanol–water (5:10:4, v/v) whereas radioactive material in the two samples of whey was extracted to an extent of about 90% with the same solvent mixture (Table I). These results suggest that radioactive hydrophilic substances—probably products of the metabolism of starter cultures—were concentrated in the whey.

The distribution of radioactivity in the various lipid fractions showed that phosphatidylcholines and phosphatidylethanolamines were the main labeled components of curd and whey. Yet, radioactive lipid fractions identified as diacylglycerols and unesterified fatty acids indicated that the glycerophospholipids added had been hydrolized to some extent. Small proportions of radioactivity were also detected in lysophosphatidylcholines. In addition, minor proportions of other labeled polar lipids derived from the added mixture of glycerophospholipids were found in both curd and whey. It is interesting to note that radioactivity in cholineglycerophospholipids and ethanolamineglycerophospholipids was markedly reduced in whey of both experiments in comparison to the composition of the added mixture of labeled phosphatidylcholines and phosphatidylethanolamines. In contrast, the radioactivity of these two labeled glycerophospholipids remained practically unchanged in curd. These findings indicate that labeled phospholipids that had been added to the cheese milk were partly changed by enzymes that may be released by starter and *Penicillium* cultures.

These results are in good agreement with the distribution of radioactivity in total polar lipids separated by two-dimensional thin-layer chromatography. Figure 1 parts a–e, shows that compared to the added mixture of radioactive phosphatidylcholines and phosphatidylethanolamines various other polar lipids are concentrated predominantly in whey.

The stereospecific distribution of radioactive acyl moieties of phosphatidylcholines and phosphatidylethanolamines of both curd and whey was studied and compared with the distribution of labeled acyl moieties of

the added glycerophospholipids from soya cells (Table II). The aim of this analysis was to find out whether or not the acyl moieties of phosphatidylcholines and phosphatidylethanolamines isolated from curd and whey were isomerized between the *sn*-1 and *sn*-2 positions of the glycerol backbone. Lipases of microbial origin catalyzing such deacylation–reacylation sequences are well-known (Pieringer, 1983). The results given in Table II show that the distribution of radioactive acyl moieties in phosphatidylcholines and phosphatidylethanolamines of curd broadly reflects that of the added glycerophospholipids indicating that these compounds remained almost unchanged during cheese making. The glycerophospholipids of whey, however, showed some minor changes in the distribution of labeled acyl moieties.

The acyl moieties of mixtures of phosphatidylcholines and phosphatidylethanolamines from curd and whey were transmethylated, and the resulting methyl esters of fatty acids were analyzed by radio gas chromatography. Methyl linoleate was the only labeled methyl ester found in the glycerophospholipids added and in those isolated from curd and whey (Table II). These findings again support the view that positional distribution as well as composition of labeled acyl moieties of phosphatidylcholines and phosphatidylethanolamines in both curd and whey remained almost unchanged.

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Thermal and Compositional Changes of Dry Wheat Gluten–Carbohydrate Mixtures during Simulated Crust Baking

I. Irving Ziderman[1] and Mendel Friedman*

A model system was designed to simulate crust baking at 215 °C for 72 min. The effect of admixtures of food carbohydrates on compositional changes and thermal stability of wheat gluten was investigated. Exothermic browning was accompanied by volatilization and nonuniform depletion of amino acids. Three transformations could be discerned: above 161 °C (internal temperature measured with a thermocouple), volatilization of nonnitrogenous compounds; above 189 °C, volatilization of nitrogenous products; above 235 °C, conversion of amino acid residues to nonvolatile nitrogenous products. The latter was the main overall fate of the protein. Above 300 °C, however, volatilization was the predominant change. Thermal reactivity of gluten–carbohydrate mixtures increased in the following order: L-ascorbic acid < potato amylose ≃ wheat starch ≃ cellulose ethers ≃ glucose ≃ fructose ≃ lactose ≃ maltose < sucrose < cellulose. The possible implications of these findings for the mechanism of browning and for baked foods are also discussed.

INTRODUCTION

In addition to its unique functional role in the formation of dough and the crumb and crust of bread and other foods, gluten is the major source of dietary protein in cereal products (Hansen et al., 1975; Betschart, 1978). The nutritional functionality of gluten may be impaired by its chemical reaction with other flour components and with baking additives, most of which are carbohydrates, including starch, the main component of flour (Block et al., 1964; Mauron et al., 1960; Gotthold and Kennedy, 1964; Jansen et al., 1964; Audidier, 1968; Palamadis and Markakis, 1980; Tsen et al., 1982, 1983).

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710.

[1]Foreign Research Associate, 1983–1984. Permanent address: Israel Fiber Institute, 91080 Jerusalem, Israel.

During baking, the mixture of water, protein, and carbohydrates in dough is exposed to two distinct transformations. Desiccation of the surface on its exposure to temperatures reaching 215 °C results in formation of a crust. The crust in turn encloses the bulk of dough in a steam phase at approximately 100 °C, forming the crumb. The nutritional impairment of gluten occurs particularly at the crust, which comprises nearly 50% of dry weight of whole bread (unpublished results). In hard biscuits, the bulk behaves thermally in a similar fashion to bread crust (Audidier, 1968).

In this study, a variety of food carbohydrates are compared in respect to their effects on the chemical stability of gluten in a model system simulating the dry conditions of crust formation. The carbohydrates investigated include widely used nutritive sugars (D-glucose, D-fructose, lactose, maltose, sucrose), vitamin C (L-ascorbic acid, sodium L-ascorbate), starches (potato amylose, wheat starch), and nonnutritive bulking agents and additives (cellulose, hy-

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