Fate of Minor Free Amino Acids and Phospholipids in Crude Tallow During Steam Splitting

Shi Rong Xu*, **Takeshi Matsuo**, **Genichi Danno**[1], **Nariaki Wakiuchi**[2] and **Satoshi Fujii**[2]
Oil & Fats Research Laboratory, Nippon Oil & Fats Co., Amagasaki, Hyogo, Japan 660

Minor free amino acids and phospholipids contained in crude tallow were monitored during steam splitting of crude tallow. The bulk of the phospholipids was found in the glycerol sidestream after splitting. Phosphatidic acid and phosphatidylcholine were present in both crude tallow and the glycerol fraction. Phosphatidylserine and phosphatidylethanolamine present in crude tallow were hydrolized with the glycerides. Because of this hydrolysis, high amounts of serine and ethanolamine are found in the fatty acid and glycerol fractions. In addition to constituent amino acids of proteins present in crude tallow, other biological amino acids such as taurine and ornithine were also present.

KEY WORDS: **Crude tallow, fatty acids, free amino acids, glycerol sidestream, phospholipids.**

Oils or fats often contain many minor components, such as free fatty acids, monoglycerides, diglycerides, unsaponifiables, tocopherols, chlorophylls, phospholipids and proteins (1). Most of these minor components are either natural constituents of oils and fats or decomposition products introduced during processing. Decomposition products of triglycerides, tocopherols, chlorophylls and carotenoids affect the oxidative and flavor stabilities of oils or fats (2–4). Moreover, organic sulphur-, phosphorus- and nitrogen-containing compounds present in oils or fats can reduce the activity of nickel catalysts used in hydrogenation of the oils or their constituent fatty acids (5–8). Such minor components are usually removed by degumming, alkali refining, bleaching and deodorization. However, even after refining, the amounts of some minor components remain at a level that necessitates an increase in metal catalyst concentration for hydrogenation. Few reports on the fate of minor components during the splitting of oils or fats have appeared in the literature. Accordingly, we studied the fate of minor free amino acids and phospholipids present in crude tallow during steam splitting.

MATERIALS AND METHODS

Hydrolysis of crude tallow. Crude tallow (1260 g) was fed into a 3-L pressure autoclave with 840 mL of deionized water. Heat and agitation were provided by the admission of live steam, causing the reaction to commence rapidly. As the reaction proceeded, a dense aqueous fraction containing the liberated glycerol separated at the base of the autoclave. Splitting was carried out at 260°C under a pressure of 52 kg/cm^2. A split of 90% was achieved after 180 min. Replacement of the glycerol fraction with 840 mL of fresh deionized water and repeating the process increased the degree of splitting to 98–99% (120 min). Glycerol fractions were combined and concentrated to 14.5% glycerol in the sidestream.

Quantitation of total phosphorus and nitrogen. The total phosphorus contents in crude tallow and in its constituent fatty acids and glycerol sidestream were determined by the molybdenum blue method (9) by means of a phosphate (KH_2PO_4) standard curve. Total nitrogen contents of the samples were determined with a total-nitrogen analytical instrument (Mitsubishikasei Co., TN-05, Tokyo, Japan) with pyridine as calibration standard.

Isolation of phospholipid fractions. To remove water-soluble constituents, five grams of stirred crude tallow were extracted with distilled water (2 × 20 mL) at 50°C for 30 min under a nitrogen stream. The crude tallow after washing was dissolved in 10 mL of chloroform and applied onto a column (2 × 55 cm) packed with 40 g of silica gel (60-mesh, K670, Katayama Chemical Co., Osaka, Japan). The eluting solvents (500 mL) used were chloroform, acetone and methanol. The dried methanol residue contained the phospholipid fraction present in crude tallow. The phospholipids in the glycerol solution (5 g) were extracted with chloroform (2 × 15 mL). The extract was reduced to 5 mL and fractionated on silica gel (40 g) as above. The methanol fraction (500 mL) eluted from the column is referred to as the phospholipids of the glycerol solution.

Thin-layer chromatography. The phospholipid fractions were spotted on silica gel G plates (0.25 × 25 × 100 mm, Analtech, Inc., Newark, DE). The plates were developed with chloroform/methanol/NH_4OH (60:35:8, vol/vol/vol). Spots were visualized by spraying the plates with Dittmer (10) reagent. Phospholipid standards (phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine and phosphatidic acid) were purchased from Sigma (St. Louis, MO).

Isolation and analysis of amino acids. Fifty grams of crude tallow or its fatty acids were added to 200 mL of distilled water, and the mixture was stirred at 50°C for 40 min under a nitrogen stream. The water layer was concentrated on a rotary evaporator under reduced pressure to 20 mL. Removal of proteins and uncharged compounds was performed successively by ultrafiltration on a disposable ultrafilter unit (UFP2LGC, 10 kDa, Millipore, Milford, MA) and by strong cation exchange chromatography on a Dowex 50 WX8 resin (40 mL, 100–200 mesh, H^+ form) (Dowex, Midland, MI) (11). The glycerol solution was extracted with chloroform to remove residual fat or its decomposition products. The amino acids in this fraction were isolated after strong cation exchange chromatography on a Dowex 50 WX8 resin (40 mL) and ultrafiltration. Analysis of the amino acids isolated from crude tallow, its fatty acids and the glycerol sidestream were conducted by high-performance liquid chromatography (HPLC) (Hitachi L8500, Tokyo, Japan). A Hitachi custom ion-exchange resin column (4.6 mm i.d. × 60 mm) (Hitachi) and 4 lithium citrate buffers (pH 2.8, 3.7, 3.6 and 4.1) were used for analysis. The flow rate was 0.35 mL/min, and the

*To whom correspondence should be addressed at Oil & Fats Research Laboratory, Nippon Oil & Fats Co., 1-56 Ohamacho, Amagasaki, Hyogo, Japan 660.
[1]Department of Agricultural Chemistry, Faculty of Agriculture, Kobe University, Nada-ku, Kobe, Japan 657.
[2]Center for Cooperative Research and Development, Kobe University, Nada-ku, Kobe, Japan 657.

eluent was monitored at either 440 nm or 570 nm after mixing with ninhydrin reagent (flow rate: 0.3 mL/min).

RESULTS AND DISCUSSION

Figure 1 gives the phosphorus and nitrogen contents for crude tallow and its fatty acid and glycerol fractions. Total phosphorus content of crude tallow was 160 ppm. However, when the tallow was hydrolyzed at high temperature and high pressure, the phosphorus content, mainly as phospholipids, was almost entirely found in the glycerol fraction (155 ppm) and the remainder in the fatty acid fraction (5 ppm). On the other hand, nitrogen-containing compounds in crude tallow behaved differently. Although minor nitrogen compounds vary with the proteins present in a given sample, the free amino acids or other nitrogen components, present in crude tallow, its fatty acids and glycerol fraction contained 330, 80 and 250 ppm of nitrogen, respectively.

The changes in phospholipids present in crude tallow as a result of steam splitting were investigated by thin-layer chromatography. Since the phosphorus in crude tallow was almost entirely shifted to the glycerol fraction after hydrolysis, phospholipids were extracted only from crude tallow and the glycerol sidestream. Figure 2 shows that nine spots in crude tallow and four spots in the glycerol solution were obtained on the silica gel plate. Comparison with phospholipid standards showed that phosphatidic acid and phosphatidylcholine are found in both the crude tallow and glycerol fraction, whereas phosphatidylethanolamine and phosphatidylserine are found only in crude tallow. It may be inferred that the latter two

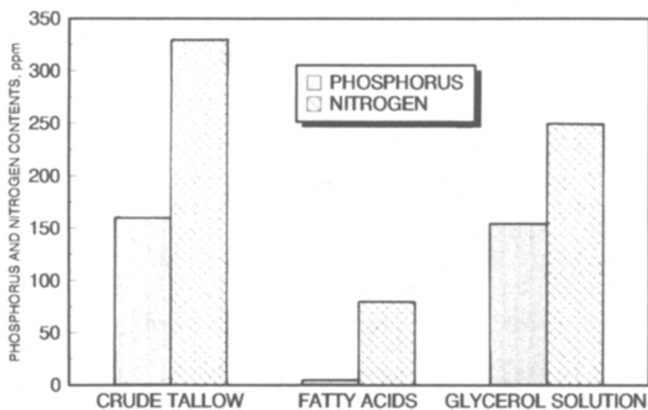

FIG. 1. Total phosphorus and nitrogen contents in crude tallow, its fatty acids and the glycerol fraction.

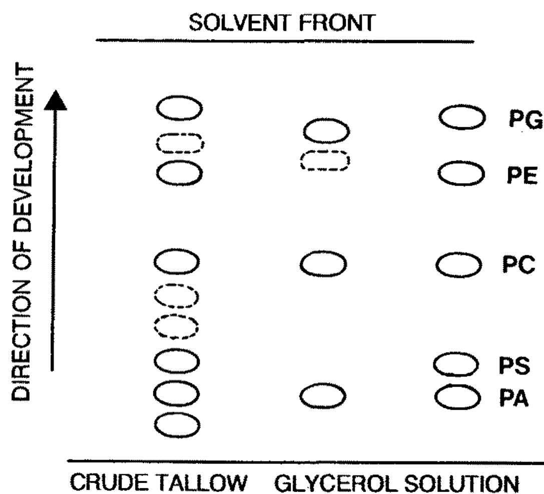

FIG. 2. Thin-layer chromatography of phospholipids obtained from crude tallow and its glycerol fraction. PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PA, phosphatidic acid.

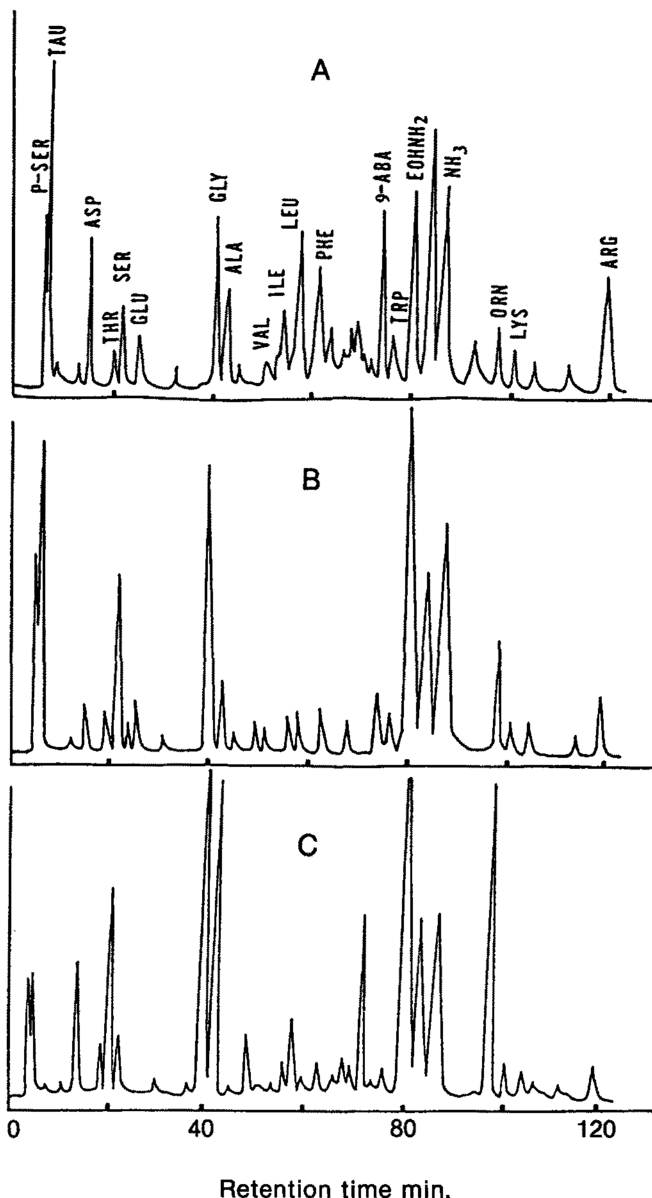

FIG. 3. High-performance liquid chromatographic analysis of free amino acids in crude tallow (A), its fatty acid (B) and glycerol solution (C).

phospholipids are hydrolyzed along with the tallow at the temperature and steam pressure used in this study.

HPLC analyses of the free amino acids obtained from crude tallow, its fatty acids and glycerol fraction were also carried out. Figure 3 gives the compositions of free amino acids in crude tallow, its fatty acids and glycerol fraction, respectively. Results indicate that crude tallow contained not only the constituent amino acids of proteins but also other biological amino acids, such as taurine and ornithine, that are mostly found in animal tissues (Fig. 3A). Compared to the content of free amino acids present in crude tallow, the amount of serine, glycine and ethanolamine are high in the fatty acid fraction (Fig. 3B). The glycerol fraction likewise contained a high content of serine, glycine and ethanolamine (Fig. 3C). Moreover, the glycerol solution also contained high concentrations of aspartic acid, alanine and ornithine. During the hydrolysis of crude tallow, aspartic acid and ornithine are partitioned into the glycerol sidestream. Both the glycerol and fatty acids fraction contained serine and ethanolamine in much higher concentration than crude tallow. This could arise from the hydrolysis of phosphatidylserine and phosphatidylethanolamine originally present in the crude tallow.

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