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Analysis of Phospholipid Classes in Various Beef Tissues by High Performance Liquid Chromatography

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ABSTRACT

An improved HPLC procedure for the separation of phospholipid classes including plasmalogens was used to determine the distributions of phospholipids in beef chuck, loin, liver, heart and kidney. The phospholipid compositions of chuck and loin were similar, whereas considerable differences in composition were observed among liver, heart and kidney. Extremely high proportions of diacyl GPC (glycerophosphocholine) and low proportions of ChoPlas (choline plasmalogen) were detected in liver compared to other tissues. The proportion of diacyl GPE (glycerophosphoethanolamine) was highest in kidney, with EthPlas (ethanolamine plasmalogen) having the highest value in chuck among the five tissues. The proportions of minor phospholipids, i.e. SGP (serine glycerophospholipid), IGP (inositol glycerophospholipid) and Sph (sphingomyelin) differed in composition dependent on the tissues. The results for plasmalogen compositions of these tissues will be useful for formulating hypotheses regarding their function.

INTRODUCTION

Phospholipids of animal tissues are almost exclusively present in biological cellular or subcellular membranes and are in a constant state of metabolic flux by synthesis and degradation. The composition of phospholipid classes in various organ tissues of vertebrates and invertebrates has been

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determined by thin layer chromatography (TLC) (Davenport, 1964; Turkki & Campbell, 1967; Rouser *et al.*, 1969; Simon & Rouser, 1969; Keller & Kinsella, 1973; de Sousa & Horrocks, 1979; Okano *et al.*, 1980; Benson *et al.*, 1983; Swietochowska *et al.*, 1983; Kester *et al.*, 1984; Diagne *et al.*, 1984), but not all the phospholipid classes, particularly ether phospholipids, in edible bovine tissues were assayed in those studies. The phospholipids play an important role in governing the quality of meat during cooking and processing (Keller & Kinsella, 1973). Plasmalogens are particularly high in bovine muscle and may have important functions in membranes (Horrocks *et al.*, 1986). They can be separated from the corresponding diacyl compounds by mild acid hydrolysis and recovery of the lyso derivatives (Renkonen, 1966; Yeo *et al.*, 1985).

The objective of this study was to observe the composition of phospholipid classes from five beef tissues (beef chuck, loin, liver, heart and kidney) by a new high performance liquid chromatographic (HPLC) separation and by phosphorus assay.

MATERIALS AND METHODS

Beef chuck eye and top loin steak (USDA choice grade), and beef liver, heart and kidney from steers fed on grain were obtained from the Kash'n Karry meat laboratory, Columbus, Ohio, at 1 day post mortem. After removal of the external fat, the tissues were homogenized with a Waring blendor, and the lipids were extracted by a modification of the method of Folch *et al.* (1957).

The HPLC used in this experiment was equipped with two pumps (Model 100, Altex Scientific Co., Berkeley, CA), an injection system with 20 μl injection loop (Model 500, Altex Scientific Co., Berkeley, CA), a column block heater (Jones Chromatography Co., Columbus, OH) operated at 33°C and a variable wavelength ultraviolet detector (LC-75, Perkin-Elmer, Norwalk, CT) set at 205 nm with full-scale absorbance of 0·64. HPLC grade organic solvents were purchased from EM Science (Gibbstown, NJ). Distilled water was purified with a MIlli-Q system plus an Organiex-Q cartridge (Millipore Co., Bedford, MA). Aqueous and organic solutions were filtered with 0·22 μm GS filters and degassed before use. The HPLC method used to separate phospholipid classes from the total lipid extracts in the five beef tissues was that of Yeo *et al.* (1985) and Dugan *et al.* (1986). The mobile phases for the HPLC were hexane/2-propanol (3:2 by vol) for solvent A, and hexane/2-propanol (3:2 by vol) with 5·5% water for solvent B. The column (4·6 mm × 25 cm) packed with Zorbax Sil was stored in hexane/2-propanol (3:2 by vol), and sufficient time was allowed for

equilibration with the solvent system until a stable baseline was obtained. For the separation of phospholipid classes except plasmalogens, the initial solvent rate was 50% B with flow rate of 1·5 ml/min, and %B was increased to 100% (duration 5 min) at 11 min and decreased to 50% B (duration 3 min) at 22 min. To separate ChoPlas and EtnPlas from the beef tissues, the CGP (choline glycerophospholipids) and EGP (ethanolamine glycerophospholipids) were collected and reacted with hydrochloric acid (Renkonen, 1966) to hydrolyze the alk-1-enyl groups. After making the lyso derivatives, corresponding to plasmalogens, by hydrolysis of the alk-1-enyl groups, diacyl GPC and 2-acyl GPC as well as diacyl and 2-acyl GPE were separated with the same HPLC solvents but with a different gradient elution. For the gradient elution to resolve the lyso compounds from their diacyl analogues, the flow ratio was programmed to give an initial composition of 50% B at 0 min, 100% B (duration 7 min) at 17 min and 50% B (duration 3 min) at 38 min with a flow rate of 1·5 ml/min.

The phospholipid classes were identified by injecting individual phospholipid standards (Sigma Chemical Co., St Louis, MO). The phosphorus of the separated phospholipids was determined quantitatively (Rouser *et al.*, 1970). The lipid samples from the beef tissues were injected three times each. The recovery of phospholipids was determined by phosphorus assays before and after injecting the lipid extracts into the HPLC column.

RESULTS AND DISCUSSION

The phospholipid class composition of beef chuck eye, top loin, liver, heart and kidney is shown in Table 1. Diacyl GPC was highest of all beef tissues in phospholipids, comprising 35·2, 35·7, 57·4, 27·1 and 29·1% in the beef chuck, loin, liver, heart and kidney, while SGP was lowest, comprising 1·7, 1·1, 4·1, 2·7 and 5·9% for the chuck, loin, liver, heart and kidney, respectively.

Beef chuck

EtnPlas (20·9%) was higher than diacyl GPE (17·3%), whereas ChoPlas (18·6%) was lower than diacyl GPC (35·2%) in the chuck. SGP was the lowest (1·7%), followed by IGP and Sph which were 2·4 and 3·9% each (Table 1).

Beef loin

The phospholipid distribution of the loin was similar to the beef chuck, but diacyl GPE (14·9%) was 2·4% less in the loin than in the chuck (Table 1).

TABLE 1
The Phospholipid Composition (Percentages of Total Lipid Phosphorus) of Five Beef Tissues[a]

Phospholipid	*Beef tissue*				
	Chuck eye	*Top loin*	*Liver*	*Heart*	*Kidney*
Diacyl GPC	35·2 ± 0·1	35·7 ± 0·1	57·4 ± 0·2	27·1 ± 0·2	29·1 ± 0·1
ChoPlas	18·6 ± 0·2	19·3 ± 0·3	2·2 ± 0·1	20·1 ± 0·1	12·5 ± 1·2
Diacyl GPE	17·3 ± 0·1	14·9 ± 0·2	16·4 ± 0·1	21·1 ± 0·1	26·0 ± 0·2
EtnPlas	20·9 ± 0·2	21·5 ± 0·1	4·9 ± 0·2	13·5 ± 0·1	8·6 ± 0·2
SGP	1·7 ± 0·1	1·1 ± 0·2	4·1 ± 0·1	2·7 ± 0·2	5·9 ± 0·1
IGP	2·4 ± 0·1	2·8 ± 0·2	8·4 ± 0·1	4·6 ± 0·1	6·5 ± 0·2
Sph	3·9 ± 0·3	4·7 ± 0·2	7·2 ± 0·1	11·0 ± 0·3	11·4 ± 0·2
Recovery (%)	99·8	98·9	99·5	99·2	99·5

[a] Phospholipid values are percentages of total lipid phosphorus and means ± standard deviation of three determinations. Alkylacyl classes are included with diacyl classes. The phospholipids were separated from lipid extracts of beef tissues by HPLC as described in the text and quantitated by phosphorus assay. The recovery of phospholipid was determined by the assay of phosphorus before and after injecting the lipid extracts into the HPLC column.

Beef liver

Diacyl GPC (57·4%) had the highest value of all phospholipid classes of the five tissues in liver and was much higher than diacyl GPE (16·4%) in the liver tissue. Both ChoPlas (2·2%) and EthPlas (4·9%) in the liver were lowest among the five tissues (Table 1).

Beef heart

Sph was present in a high proportion (11·0%) in this tissue when compared to the skeletal muscle (chuck and loin) and liver. The highest amount of ChoPlas was in the heart at 20·1% (Table 1).

Beef kidney

This tissue was characterized by the highest amounts of diacyl GPE and Sph (26·0 and 11·4% of the entire phospholipid composition) from the five tissues. A much higher level of ChoPlas and EtnPlas was observed in the kidney than in the liver (Table 1).

Generally, marked differences in the phospholipid composition among liver, heart and kidney, but no major differences in the composition between the chuck and loin, were noted in this experiment. Except for liver which had

extremely low ChoPlas (2·2%), the ChoPlas concentrations in the other tissues accounted for 12·5 to 20·1%. The variation of EtnPlas among the tissues was higher than that of ChoPlas. After diacyl GPC, the proportion of diacyl GPE was highest in the liver, heart and kidney. SGP had the lowest proportion of the phospholipid classes in all tissues. IGP was in the range of 2·4% (chuck) to 8·4% (liver), whereas Sph was lowest in the chuck (3·9%) and considerably high in the heart (11·0%) and kidney (11·4%).

Reports of phospholipid composition of bovine tissues include *longissimus dorsi* muscle (Davenport, 1964), kidney and liver (Rouser *et al.*, 1969), and heart and skeletal muscle (Simon & Rouser, 1969). Davenport (1964) showed much lower composition of EtnPlas and ChoPlas than this study, but Rouser *et al.* (1969) did not report any plasmalogen of bovine tissues. Diagne *et al.* (1984) studied the differences in phospholipids among brain, heart, kidney and liver from the three different mammalian species (human, rat and guinea pig). Okano *et al.* (1980) compared the phospholipid distributions of white, intermediate, red and heart muscle in rat and found higher percentages of plasmalogens (alkenylacyl type) in EGP than in CGP for those muscles in agreement with this study. The plasmalogen proportions from various organs and animal species were reviewed by Horrocks & Sharma (1982). These researchers mentioned above used various TLC methods with different conditions, which might cause variations of the phospholipid composition. Several other factors also affect the phospholipid values, such as post-mortem enzymatic degradation, methods for lipid extraction, reproducibility of the phospholipid class analysis and species variability (Rouser *et al.*, 1969). Though some amount of DPG (diphosphatidyl glycerol) was shown in other studies, it was not found in this study. DPG might not be detected in the study because of different sample conditions with other studies or problems in DGP resolution of this HPLC method. Generally it is difficult to obtain the desired reproducibility and consistency by TLC. However, the new modified HPLC method deployed for this study overcomes the problems with TLC and separates major phospholipid classes efficiently.

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