MOLECULAR SPECIE COMPOSITION OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE IN SKELETAL MUSCLE OF FARM ANIMALS.

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SUMMARY

We have developed a rapid and convenient reversed-phase HPLC method for the separation of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) molecular species. The main molecular species were eluted in 30 min on Lichrosorb ODS phase column using a mobile phase composed of methanol/hexane/ammonium acetate (100/6/0.1M). The species were quantified by an evaporative light scattering detector. In the PE, plasmalogen molecular species accounted for 35-64% whereas in the PC they ranged from 8 to 20%. The main molecular species of PE plasmalogens were 16:0/18:2, 16:0/20:4 and 18:0/20:4 while those of PC were 16:0/18:1, 16:0/18:2 and a slight amount of 16:0/20:4. The main diacyl species of PE were 18:0/18:2 and 18:0/20:4 while 16:0/18:1 and 16:0/18:2 were the most abundant ones of diacyl PC. It is noteworthy that most of the molecular species containing long chain PUFA are in PE plasmalogens. This observation should explain the high sensitivity of PE to oxidation.

Introduction

The phospholipids are the main components involved in oxidation of meat and meat products. It is largely accepted to ascribe the sensitivity of phospholipids to oxidation to their high amount of polyunsaturated fatty acids (PUFA). Thus, phosphatidylethanolamine which exhibit a high proportion of long chain PUFA oxidized faster than phosphatidylcholine which contain only a small amount of these fatty acids (Keller and Kinsella, 1973 ; Gandemer, 1990). However the mechanisms involved in phospholipid oxidation is not yet clearly understood. One of the main problem is related to the fact that phospholipids are formed of a large number of molecular species which could be distinguished by the polar head and the fatty chains. It is obvious that the propensity of the various phospholipid molecular species to oxidize is largely related to the type of polar head and fatty chains. For a better understanding of the mechanisms of phospholipid degradation, it is interesting to quantify individual molecular species of the main phospholipid classes. In the present work, we have determined the molecular specie composition of phosphatidylethanolamine and phosphatidylcholine of a glycolytic muscle of beef, rabbit, pork and turkey.

Material and methods

Phospholipid extraction and fractionation : Lipid were extracted from *Longissimus Dorsi* of beef, pork and rabbit and *Pectoralis* of turkey according to the Folch *et al.* (1957) procedure. The phospholipid fraction was purified from total lipid extracts on silica cartridges (Sep-Pak, Waters) according to the method described by Juaneda and Rocquelin (1985). Phospholipid classes were separated by semi-preparative HPLC using a Gilson binary delivery system and a rheodyne valve injection equipped with a 200 ml loop. The column (300 mm long x 7.5 mm i.d.) was packed with 5 mm Lichrospher silica (Si-60, Merck). Phospholipids were detected with a light scattering detector (DDL 10, Cunow) paired with suitable computer and software. The main phospholipid classes were eluted in 45 min using a gradient mode according to Leseigneur *et al.* (1989) procedure. The eluent A was pure $CHCl_3$. The eluent B was a mixture of $CH_3OH/H_2O/NH_4/CHCl_3$ (92/5/2/1). All solvents were filtered through a 0.45 mm silicon filter (Millipore). Each class was collected manually using a splitter set between the end of the column and the detector. The solvent was evaporated under vacuum and the phospholipid was redissolved in 1 ml of methanol/hexane for further reversed-phase analysis.

Fatty acid and fatty aldehydes composition : Fatty acid and fatty aldehyde composition of PE and PC was determined by Gas Liquid Chromatography of methyl esters and dimethyl acetals prepared as described by Berry *et al.* (1965). The analysis was carried out using a Hewlet Packard 5890 equipped with a flame ionization detector and a on-column injector. Hydrogen was the carrier gas and the temperature of the detector was 250°C. The analysis was performed on a capillary column (30 m. long x 0.32 mm i.d.) containing a polar stationary phase (Superox, Alltech). The oven temperature was held at 40°C for 1 min and then increased at 15°C/min to 200°C where it was maintained until the end of the analysis. Methyl esters and dimethyl acetals were identified using gas chromatography-mass spectrometry (HP MSD 5971).

Molecular species separation and quantification : PE and PC were separated into molecular species on Superspher RP18 column (250 mm long x 4.5 mm i.d.) packed with 4 mm particles. The mobile phase was methanol/hexane/0.1M ammonium acetate (100/6) and the flow rate was 1 ml/min. The molecular species were quantified with an evaporative light-scattering detector as described above. For most phospholipid molecular species, the detector response was linear in the range of 50 to 200 mg but tended to tail off rapidly below 10 mg (Stolyhwo *et al.*, 1987). Thus results were expressed as percentage of sum of area of molecular species.

Results and Discussion

Fatty acids and aldehydes composition : In the four animal species, the fatty acid composition of PC showed large similarities. Thus, the relative proportions of saturated, monounsaturated and polyunsaturated fatty acids (PUFA) were 33.3-40.8%, 23.5-38.7% and 24.5-37.8% respectively. The main fatty acids of PC were palmitic (23.2-30.8%), oleic (22.0-35.8) and linoleic (14.8-30.7). However, beef PC contained less PUFA than those of the three others species. This result is related to the low proportion of N-6 PUFA in beef PC as compared to those of rabbit, pork and turkey (19.5% instead of 33.1-37.8%). Dimethyl acetals accounted for 3-8% of the fatty acid methyl esters whatever the animal species. The major aldehyde was palmityl aldehyde which accounted for almost 84 to 88% of the total fatty aldehyde.
As compared to PC, PE contained more PUFA and more aldehydes (54.8-60.1% versus 24.5-37.8% and 26.6-42.5% versus 2.4-8.4%, respectively). Within the four animal species, the respective amounts of saturated, monounsatured and polyunsaturated fatty acids were close (16.3-27.7%, 13.6-28.8%, 54.8-60.1% respectively). The higher proportion of N-3 PUFA were found in beef and turkey PE (15.4-28.5%) whereas these fatty acids accounted for only 5.5 and 6.7% in rabbit and pork respectively. Pork PE contained the highest proportion of N-6 PUFA and beef PE the lowest (51.6 versus 31.4%). These data are in good agreement with those obtained for PC and PE from rabbit and chicken muscles by Marai and Kuksis (1973) and from pork, beef and chicken meats by Fogerty et al. (1991).

Molecular species of PC and PE : Whatever the animal species, the main molecular species of PC were the diacyl species 16:0/18:1 and 16:0/18:2. In beef PC, the amount of the 16:0/18:1 was far higher than that of the 16:0/18:2 (38.4 versus 15.6%) while opposite results were observed in other animal specie PC (23.7-28.2% for 16:0/18:1 versus 33.4-48.1 for 16:0/18:2). The 18:0/18:2 specie was present in significant amount in beef and turkey PC (8 - 13%) while it was very low in rabbit and pork PC (2-4%). Pork and rabbit contained less alkenyl acyl species (13 and 8% respectively) than beef and turkey (19.5 and 18.2% respectively). The most abundant alkenyl acyl was 16:0 ald/18:2 (4.1 to 10.9%). The 16:0 ald/18:1 accounted for 1.8 to 8.7% according to the animal species. Long chain PUFA were found in both diacyl and alkenyl acyl species of beef, rabbit and turkey PC while they were in diacyl species of pork PC.
The main diacyl species of PE were 18:0/20:4 and 18:0/18:2 in nearly equal proportions, except in rabbit. The PE of beef contained a significant amount of 16:0/18:2 (9.1%). The 16:0/18:1 and 18:1/22:6 represented 2.2 to 5.6% and 2.7 to 4.2% of the total molecular species, respectively. The alkenyl acyl species ranged from 35.9% in beef PE to 64.4% in rabbit PE. Marked differences were observed in the relative proportions of the main alkenyl acyl species between animal species. In rabbit PE, the most abundant alkenyl acyl specie was the 18:0 ald/20:4 (38.3%) followed by the 18:0 ald/18:2 (12.8%). In the other animals, the main alkenyl acyl species of PE were the 18:0 ald/20:4 (6.0 to 16.3%), the 16:0 ald/20:4 (6.1 to 17.6) and the 16:0 ald/18:2 (8.2 to 12.8%). The 16 ald/22:6 specie was found in turkey, beef and rabbit PE (4.6 to 10.3%) but it was not detected in pork PE. The 18:0 ald/20:4 was present in a significant amount in beef and pork PE (6.5-7.9%) but it was very low in rabbit and turkey PE (less than 2%). Pork PE contained 8.8% of the 18:1 ald/18:2 specie while it accounted for less than 3% in PE of other animals. Very little information on molecular specie composition of PE and PC of skeletal muscle is available in the literature (Marai and Kuksis, 1973 ; Fogerty et al., 1991). The results obtained in different laboratories are difficult to compare because the authors have used various methods and

the data were not expressed on the same basis. However, all these studies indicated that a large amount of muscle long chain PUFA is combined with aldehydes in PE alkenyl acyl species. In recent studies, Fogerty et al. (1989, 1990) have shown that plasmalogen were hydrolysed and a part of the PUFA were lost in PE during heating of various meats. In this respect, it seems that PE alkenyl acyl molecular species should be the main substrate involved in phospholipid alteration during meat processing. At present very little is know on the behaviour of the PE plasmalogen during meat processing. The present method should be a useful tool for the study of the role of these lipids in meat quality.

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