Effect of different mechanical and heat procedures and storage conditions on the content of individual phospholipids in pork

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In our previous papers (5,6,7) we examined different mechanical and heat procedure and storage condition effects on total phospholipid content changes, as well as their share in oxidation and hydrolysis of total lipids.

We identified nine phospholipids and established their amounts (5), as well as the contents of four unidentified phospholipids. Thereby we isolated more phospholipids than those refered to in the available literature. Within our examinings we isolated the phosphatidic acid, glycerophosphatidylcholine as well as four unidentified components, amount of which remains unmentioned in the available literature, at least as far the red meat is concerned.

The objective of this work was to examine how certain mechanical and heat procedures and storage conditions, interesting for industrial and culinary practicies, effect the individual phospholipid content changes.

Material and methods

For this examination M.longissimus dorsi of the domestic white meaty pigs (females, 6 months old, 75-85 kg in dressed weight, equally fed at the same farm) was used. Having cooled the halves for 24 hours at 2-4°C, muscle parts from the region covering last two thoracic and first three lumbal vertebrae, were taken, the visible fatty and connective tissues beeing then removed from them. Two ways of mechanical treatment were applied: intensive grinding, namely preparation of homogenates and meat cutting into pieces (meat slices as thick as vertebrae were devided into four parts by two cross-cuts), whereby 10% of 20% sodium chloride water solution was added, namely injected. The following heat treatments were used: pasteurization (200 g cans were kept in water at 80°C for about 30 minutes till 70°C in the can content centre obtainment), sterilization (118°C/30 min.; maximum temperature in the can content centre - 115°C) and roasting (muscle pieces being previously rubbed in with sodium chloride - 1% on meat weight - were roasted in dish-shaped aluminium foils in drying oven at 180--200°C till the obtainment of 65-70°C in the meat centre).

Fresh meat and treated samples were examined immediately after mechanical and heat procedures and 3,6,9,12 and 15 days after storage at 2-3°C and 30, 60, 90, 120, 150 and 180 days after storage at - 18°C. The surface layer, 8 mm in width, and the central part of roasted meat were analysed separately.

Total lipids were extracted (8) from 40 g-ground sample. Prior to being applied on thin layer, phospholipids were purified by column chromatography, using acid-treated and activated Florisil of 100-200 mesh (2). Before elution, Florisil in column was rinsed with 40 ml portions of acetone and methanol and with 100 ml of chloroform. After applying the total lipid sample, being previously evaporated and dried by chloroform, phospholipids were extracted by successive running of 100 ml of chloroform and 70 ml portions of chloroform-methanol mixtures in combinations of 9:1, 8:2, 7:3, 6:4, 4:6, 3:7, 2:8 and at the end 100 ml of methanol (the combinations of chloroform-methanol mixtures were selected on the basis of phospholipid chromatograms in the mentioned fractions). After evaporation in the rotation vacuum evaporator and drying in vacuum, the samples were transferred into a 50 ml volumetric flask. Aliquots for the determination of phosphorus content (16), namely phospholipid content (P x 25) as well as aliquots for separation of phospholipids on thin layer were taken.

0.15 ml aliquots were placed upon plates (20 x 20 cm) coated with a 0.3 mm layer of Silikagel G, buffered with borate buffer (1) and activated at 110°C for 1 hour. Chloroform-methanol-30% ammonia-water (140:50:7:3, by vol.%) and chloroform-methanol-acetic acid-water (160:20:4:1,5, by vol.%) (13) were used as solvents for the development of chromatograms. The spots were detected by iodine vapour and separated phospholipids were identified through Rf values, by comparison with the standards. The contents of individual phospholipids were determined (15) after the spots had been scrapped from the plates.

Results and discussion

Table 1 presents the content of individual phospholipids, expressed in mg%P, mean values in fresh meat and samples after mechanical treatment, heat treatment as well as storage at 2-3°C for 15 days and at -18°C for 180 days.

Mean values of the individual phospholipid contents, obtained immediately after mechanical meat treatment, in raw homogenate and in raw meat pieces, indicate that a considerable increase of the contents of glycerophosphatidylcholine, lysophosphatidylcholine and components at the start X_s follow the decrease of quantitatively mostly represented phospholipids: phosphatidylcholine (10.01 in the homogenate and 8.68% in meat pieces) and phosphatidylethanolamine (14.39% in the homogenate and 11.43% in meat pieces) (Table 2). There have been observed a considerable increase of unidentified phospholipid X_3, certain increase of phosphatidylinositol, but a decrease of sphingomyelin and cardiolipin quantities, and then also of the unidentified X_2 as well as a quantity of lipid phosphorus in the total quantity of phosphatidylserine and phosphatidic acid. Since these two components were not separated by two-step one-dimensional chromatography on thin layer, two-dimensional chromatography was applied, showing a constant increase of phosphatidic acid and a decrease of phosphatidylserine contents. Therefore, the decrease of their total quantity indicates that the increase of phosphatidic acid was lower than the decrease of phosphatidylserine.

Changes of the content of both total and individual phospholipids are more expressed in the homogenate than in meat pieces. These results point out a considerable tissue desintegration effect, and, consequently, a higher possibility of hydrolytic and oxidative change appearances due to larger surfaces being exposed to the effect of air, namely oxygen in the case of intensive grinding.

A considerable increase of lysophosphatidylcholine and glycerophosphatidylcholine quantities as well as of components at the start and decrease of the contents of phosphatidylcholine, phosphatidylethanolamine and others, during storage of raw samples, are probably the result of the effect of phospholipases able to act even at -18°C (4,9). Components at the start are products of phospholipid desintegration and probably certain unidentified phospholipids.

Heat procedures of the homogenate and meat pieces - pasteurization and sterilization, cause higher decrease of the content of total phospholipids than mechanical treatments but lower individual phospholipid content changes. A higher percentage decrease of phospholipid contents, both total and individual, was observed in homogenates than in meat pieces heat treated in the same way, being higher during sterilization than during pasteurization, whereas during both ways of storage these differences were smaller. Higher increase of free fatty acid content both in meat pieces and raw homogenate, in relation to heat treated samples, during and at the end of storage, that was found in our previous examinations (5,6,7), could be ascribed to the lipase activity. The increase of the content of lysophosphatidylcholine and components at the start could be caused by phospholipases and/or by hydrolysis under the heat effect (11).

Comparing sterilized and pasteurized meat piece samples as well as sterilized and pasteurized homogenate ones, it can be seen that meat roasting causes higher phospholipid decrease in the surface layer than mechanical treatment and pasteurization together, sterilization respective-

Mean values of individual phospholipid contents in samples after mechanical and heat procedures as well as storage[1]

Table 1

Sample	Days	x_3^*	Phosphatidylserine and phosphatidic acid	Phosphatidylinositol	Glycerophosphatidylcholine and lysophosphatidylcholine	Sphingomyelin	Phosphatidylcholine	Phosphatidylethanolamine	Cardiolipin	x_1^*	x_2^*	x_3^*
		Phospholipids, in mg% p (expressed on dry matter)										
Fresh meat		0.798	6.842	1.878	1.153	4.543	44.261	22.288	4.195	0.653	0.742	2.091
Mechanical treatment												
a. Homogenate	0	1.670	6.119	1.984	1.679	3.918	39.830	19.080	3.757	0.611	0.704	2.892
	15	5.596	5.543	1.601	9.407	2.854	21.589	11.920	3.129	1.633	0.669	3.785
	180	4.995	4.889	1.282	7.280	2.503	16.970	10.000	2.789	1.461	0.616	3.326
b. Meat pieces	0	1.152	6.069	1.987	1.565	4.377	40.420	19.740	4.016	0.654	0.688	3.019
	15	5.483	5.876	1.713	8.355	3.261	22.388	11.818	3.502	1.397	0.667	3.640
	180	5.360	5.039	1.407	7.682	2.580	18.058	10.250	2.768	1.224	0.526	3.037
Heat treatment												
1. Pasteurization												
a_1. Homogenate	0	2.072	6.140	1.830	3.212	3.777	37.448	18.196	3.902	0.699	0.757	2.870
	15	4.245	5.340	1.506	6.546	2.758	22.830	12.173	3.390	1.324	0.606	2.804
	180	3.580	4.341	1.266	6.724	2.453	17.120	9.720	2.665	0.990	0.436	2.202
b_1. Meat pieces	0	1.447	6.083	2.072	2.380	4.152	39.230	19.046	3.621	0.693	0.933	3.099
	15	3.898	5.307	1.487	6.337	2.980	23.426	12.016	3.038	1.278	0.782	2.900
	180	3.505	4.692	1.176	6.039	2.280	19.540	9.925	2.639	1.143	0.337	2.523
2. Sterilization												
a_2. Homogenate	0	2.135	6.198	1.780	3.733	3.453	36.808	17.700	4.095	0.676	0.791	2.975
	15	3.795	5.120	1.427	6.554	2.464	22.842	12.166	3.258	1.203	0.557	2.765
	180	3.245	4.171	1.271	5.123	1.942	18.636	9.970	3.006	1.154	0.575	2.474
b_2. Meat pieces	0	1.637	6.111	1.933	3.249	3.882	38.368	18.433	3.536	0.827	0.827	3.249
	15	3.757	5.184	1.587	6.125	2.688	23.328	12.122	2.918	1.261	0.614	2.797
	180	3.251	4.293	1.296	5.681	2.252	19.208	10.109	2.403	1.139	0.545	2.365
3. Roasting												
c_1. Surface layer	0	2.259	5.236	1.578	3.775	3.142	33.226	16.007	3.089	0.703	0.763	2.685
	15	3.522	4.452	1.332	6.456	2.274	22.284	11.538	2.646	0.858	0.618	2.478
	180	3.235	3.885	1.125	5.619	1.878	19.004	9.773	2.286	0.722	0.511	2.193
c_2. Central part	0	2.157	6.027	1.648	3.773	3.410	39.490	17.388	3.305	0.784	0.840	2.941
	15	3.975	5.254	1.325	6.246	2.413	23.630	12.288	2.771	0.998	0.883	2.733
	180	3.286	4.401	1.166	5.467	1.989	19.317	10.157	2.364	0.992	0.590	2.385

[1] after storage at 2-3°C for 15 days, namely at - 18°C for 180 days

* unidentified phospholipids

Phospholipid content changes in samples due to mechanical and heat procedures as well as storage conditions

Table 2

Treatments	Phospholipid content* decrease, % (expressed on dry matter) Storage days								
	0			15 days at 2-3°C			180 days at -18°C		
	TP	PC	PE	TP	PC	PE	TP	PC	PE
Mechanical treatment									
a. Homogenate	1.74	10.01	14.39	23.10	45.80	37.53	33.81	57.39	47.59
b. Meat pieces	0.87	8.68	11.43	22.06	44.61	40.13	33.82	55.32	48.07
Heat treatment									
1. Pasteurization									
a_1. Homogenate	4.04	5.98	4.63	20.83	39.03	33.10	35.09	54.28	46.58
b_1. Meat pieces	2.16	2.94	3.51	22.91	40.28	36.91	34.74	50.19	47.89
2. Sterilization									
a_2. Homogenate	4.80	7.58	7.23	21.04	37.94	31.26	34.11	49.37	43.67
b_2. Meat pieces	3.52	5.08	6.62	22.87	39.20	34.24	35.10	49.94	45.16
3. Roasting									
c_1. Surface layer	13.14	24.93	28.18	20.50	32.93	27.92	32.01	42.80	38.94
c_2. Central part	7.33	10.78	21.98	19.92	40.16	29.33	34.05	51.08	41.58

TP - total phospholipids

PC - phosphatidylcholine

PE - phosphatidylethanolamine

*Mean value of total phospholipid content in meat was 0.585%, and expressed on dry matter - 2.291%.

ly (Table 2). A considerably higher phospholipid content decrease in the surface layer of roasted meat, that is to say during roasting, is probably the result of oxidation, hydrolytic desintegration, browning reaction and lipid and protein copolymerization development (12). In favour of this proves the highest average reduction of quantitatively most represented phospholipids, phosphatidylcholine and phosphatidylethanolamine in the surface layer of roasted meat and that is probably why the oxidative changes are most expressed in it. In relation to the surface layer of roasted meat, immediately after heat treatment this decrease is lower by 3.5 times in sterilized homogenate, 4.5 times in sterilized meat pieces, 5 times in pasteurized homogenate and 8 times in pasteurized meat pieces. The highest increase of lysophosphatidylcholine content was in roasted meat, and then follow sterilized homogenate, sterilized meat pieces, pasteurized homogenate and, finally, pasteurized meat pieces.

During the storage of all heat treated samples as well as of raw, mechanically treated ones, in addition to the increase of glycerophosphatidylcholine, lysophosphatidylcholine and components at the start, the decrease of the contents of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, cardiolipin and phosphatidylinositol is also observed, although these two latter ones are with certain irregularities during different storage periods (they were not presented due to a shortage of space). However, during storage of pasteurized pieces and raw samples, the increase of the quantity of phosphatidylinositol was observed. Phosphatidic acid content is constantly beeing increased, and the one of phosphatidylserine decreased. Their total content is being decreased during sample storage and roasting, whereas it is being increased during sterilization and pasteurization, indicating inevitably that in this case the increase of phosphatidic acid quantity is considerably higher than phosphatidylserine content decrease.

In our previous examinations (5,6,7) we have concluded that during mechanical treatment, heat treatment and storage, 72 to 89% of the obtained peroxide values, namely 85 to 94% of TBA numbers originate from phospholipids, and from the total value of free fatty acids - 65 to 73% come from phospholipids.

Examining the influence of phosphatidylethanolamine and phosphatidylcholine in model-samples, Igene and Pearson (10) concluded that phosphatidylethanolamine mostly contributed to rancidity development, and Corliss and Dugan (3) as well as Tsai and Smith (14) pointed out that phosphatidylethanolamine expressed a considerably stronger prooxidative effect than phosphatidylcholine.

Following the phospholipid content changes as well as observing taste panel results we consider that phosphatidylethanolamine and phosphatidylcholine changes are anyway significant for rancidity development, beeing pointed out by the above mentioned authors too, but, on the other hand, the effect of other phospholipids must not be neglected either.

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