Der Gehalt der Phospholipiden im M. longissimus dorsi der Schweine und der Einfluss von verschiedenen mechanischen und thermischen Behandlungsverfahren auf die an ihnen entstandenen Veränderungen

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Der Gehalt der gesamten und einzelnen Phospholipiden im M. longissimus dorsi von Schweinen sowie der Einfluss des mechanischen und thermischen Behandlungsverfahren auf den Gahalt der gesamten Phospholipiden und ihre hydrolitische und oxidative Veränderungen wurde bestimmt. Es wurden zwölf Phospholipiden auseinandergestrent und neun davon identifiziert. Der Grad der Fleischstrukturdesintegration ist von der entscheidenden Bedeutung für die Lipidenveränderung. Die Verminderung des Phospolipidengehalts und die Vergrösserung des freien Fettsäuerenglhalts und oxidativen Lipidenveränderungen, sind am ausgeprägsten in dem gebratenen Fleisch. Die Sterilisation bedingt grössere Veranderungen von Phospolipiden- und der freien Fettsaueregehalts als die Pasterisation. Die oxidative Veränderungen sind stärker ausgeprägt bei der Fleischpasterisation. Der prozentuelle Anteil von Phospholipiden in den oxidativen und hydrolitischen Lipiden-Veränderungen wurde ebenfalls festgestellt.

Phospholipid content in M. longissimus dorsi of hogs and influence of different procedures of mechanical and heat treatments on phospholipid changes

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The content of total and individual phospholipids in M. longissimus dorsi of hogs as well as the influence of different mechanical and heat treatments of meat on the content of total phospholipids and on their hydrolytic and oxidative changes were examined. Twelve phospholipids were separated, nine of them having been identified. The degree of meat structure desintegration is decisive for lipid changes. The decrease of phospholipid content and the increase of free fatty acid content and oxidative changes of lipids, especially phospholipids, were pronounced to the highest extent in roasted meat. In relation to pasteurization, sterilization causes higher changes of phospholipid and free fatty acid contents. Oxidative changes were more markedly expressed in meat pasteurization. The percentage of phospholipid participation in oxidative and hydrolytic changes of lipids was established.

J 5:2

Teneur en phospholipides dans le M. longissimus dorsi des porcs et effet de divers traitements mécaniques et thermiques sur leurs modifications

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La teneur en phospholipides dans le M. longissimus dorsi de porcs et l'effet des traitements mécaniques et thermiques sur la teneur des phospholipides et leurs modifications hydrolithiques et oxydatives sont examinées. On a separé douze phospholipides et identifié neuf. Le degré de désintégration de la structure de la viande est decisif pour les modifications examinées des lipides. Le décroissement de la teneur en phospholipides et l'accroissement de la teneur en acides gras libres et de modifications oxydantives des lipides, surtout des phospholipides , sont les plus marquées dans la viande rotie. La stérilisation a pour effet un plus grand changement de la teneur en phospholipides et en acides gras libres que la pasteurisation. Les modifications oxidatives sont plus marquées que dans la viande pasteurisée. On a constaté la part en pourcent des phospholipides dans les modifications oxydatives et hydrolitique des lipides.

Содержание фосфолипидов в M. longissimus dorsi свиней и влияние различных способов механической и термической обработки на их изменение

ВЕСЕЛИНКА ДЖОРДЖЕВИЧ, ФРАНЦ БУЧАР, ИВАНА ДЖУИЧ и НАДА РАДОВИЧ
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Утверждено содержание всеукупных и поодиночных фосфолипидов в M. longissimus dorsi свиней. и испитано влияние способа механической и термической обработки мяса на содержание всеукупных фосфолипидов и их гидролитические и оксидативные изменения. Разлучено двенадцать, а индентифицировано девять фосфолипидов.Степень дезинтеграции структуры мяса является самым решающим для изменения липидов.Уменьшение содержания фосфолипидов, повышение содержания свободных жирных кислот и оксидативных изменений липидов, а больше всех фосфолипидов, являются самым выразительным в жаренном мясе. Стерилизация обусловливает большие изменения содержания фосфолипидов и свободных жирных кислот чем пастеризация. Оксидативные изменения сильнее выражены при пастеризации мяса. Утверждено процентуальное участие фосфолипидов в оксидативных и гидролитических изменениях липидов.

Phospholipid content in M. longissimus dorsi of hogs and influence of different procedures of mechanical and heat treatments on phospholipid changes

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Introduction

There is a lack of data on the content of individual phospholipids in meat (1, 8, 10,15,16, 20, 24, 27). Influence of mechanical treatment on the changes of phospholipids both in raw meat and in heat treated meat has been insufficiently studied (3,11,12,14,15,17,22,26). In addition, in the available literature we did not find data on the influence of intensive meat grinding rate on phospholipid changes.

The present study was undertaken to establish the content of total and individual phospholipids in M. longissimus dorsi of domestic hogs, to determine the effect of mechanical treatment and different ways of heat treatment on the content and hydrolysis and oxidation of phospholipids and to determine the participation of phospholipids in lipid hydrolisis and oxidation in mechanically treated meat and in heat treated meat.

Materials and Methods

M. longissimus dorsi of domestic white meaty hogs (females, 6 months old, 75-85 kg in dressed weight, deriving from the same farm and fed in the same way) was used in this study. After cooling of sides at 2-4^{o}C for 24 h, sections covering the last two thoracic and the first three lumbar vertebrae were taken from muscles of approximately the same pH value. They were carefully freed of obvious agregates of connective and adipose tissues. Two ways of mechanical treatment were applied: intensive grinding rate, namely preparation of homogenates, and cutting into pieces (meat slices as thick as vertebrae were cut into four equal parts). On that occasion, 20% NaCl-water solution (-12^{o}C) was added or injected in the quantity of 10%.

The following heat treatments were applied: pasteurization (200 g cans were kept in water at 80^{o}C for about 30 minutes till the obtainment of 70^{o}C in the can content center), sterilization (118^{o}C for 30 minutes, maximum temperature in the can content center - 115^{o}C), and roasting (meat rubbed in with NaCl - 1% on meat weight - in dish-shaped aluminium foils, were roasted in drying oven at 180-200^{o}C till the obtainment of 65-70^{o}C in meat center).

Fresh meat and samples obtained after mechanical and heat treatments and cooling were immediatelly examined. The surface layer, 8 mm in width, and the central part of roasted meat were analysed separately. Each experimental group covered six samples.

Total lipids were extracted (7) from 40 g-ground sample; 12 mg of dodecylgallate was added. Neutral and polar lipids were separated on acid-washed Florisil (4) columns with portions of 300 ml methanol and 300 ml chloroform. Before elution, Florisil in column was treated with aceton, methanol and chloroform. The content of total, polar and neutral lipids, as well as peroxide value (19) (PV), TBA number (23,25) (TBA) and free fatty acid content (19) (FFA) in them, were determined. Before other determinations, total lipids were placed on columns prepared in the same way, except that in the first case Florisil of 60-100 mesh was used, and in the second case Florisil of 100-200 mesh. Elution was accomplished by 100 ml chloroform, 70 ml-portions of chloroform-methanol in combinations 9:1, 8:2, 7:3 6:4, 4:6, 3:7, 2:8 and, at the end, by 100 ml methanol. Phospholipid content (PL) was calculated from the P-content (Px25), which was determined (30) in aliquots taken from 50 ml-lipid sample in chloroform 0,15ml aliquots were applied on plates (20x20 cm) coated with a 0,3 mm layer of Silikagel G, buffered with borate buffer (2) and activated at 100^{o}C for 1 h. The developing solvents were chloroform-methanol-30% aq ammonia-water (140:50:7:3, by vol.) and chloroform-methanol-acetic acid-water (160:20:4:1,5 by vol.) (18). The spots were detected by

iodine vapour, and PL fractions were identified by comparison with reference compounds, The contents of individual PL (in mg%P) were determined (28) after the spots were scrapped from the plates.

Mean value, standard deviation and variance coefficient were determined. Influence of mechanical and heat treatments on the analysed changes of lipids, namely PL, were examined by the t-test analysis of differences (21).

Results and Discussion

The examination results of total and individual PL are presented in table 1. The content of PL in the examined part of M. longissimus dorsi of domestic white meaty hogs was 0.585%, and their participation in total lipids was 20.31% on average. This value is very close to the quantity established by Keljman (13) and by Wood and Lister in M. longissimus dorsi of Large White hogs but considerable lower than the quantity of 0.633% established by Šmidt and Ljaskovskaja in the same muscle of bacon hogs (20).

Content of total lipids and phospholipids as well as individual phospholipids in M. longissimus dorsi of hogs

Table 1

	$\bar{x}$	s	Cv	$\bar{x}_{DM}$	In.Pl/Pl
Total lipids, %	2,88	0.33	11,46	11.28	-
Total phospholipids, %	0.585	0.013	2.26	2.291	-
Individual phospholipids , mg%P					
X_s^*	0.204	0.013	6.55	0.798	0.89
Phosphatidylserine and phosphatidic acid	1.747	0.050	2.85	6.842	7.65
Phosphatidylinositol	0.480	0.019	4.06	1.878	2.10
Glycerophosphatidylcholine and lysophosphatidylcholine	0.294	0.010	3.41	1.153	1,29
Sphingomyelin	1.160	0.091	7,89	4.543	5.08
Phosphatidylcholine	11.301	0.419	3.70	44.261	49.47
Phosphatidylethanolamine	5.692	0.155	2.72	22.288	24.92
Cardiolipin	1.072	0.057	5.28	4.195	4.69
X_1^*	0.166	0.013	7.58	0.653	0.73
X_2^*	0.190	0.013	6.61	0.742	0.83
X_3^*	0.534	0.025	4.66	2.091	2.34

$\bar{x}$ - mean value
s - standard deviation
Cv - variance coefficient
$\bar{x}_{DM}$ - mean value, quantity espressed on dry matter
* - unidentified phospholipids
In.Pl/Pl - individual in total phospholipids, %

A total of 9 PL was identified. Phosphatidylserine and phosphatidic acid as well as glicerophosphatidylcholine and lysophosphatidylcholine could not be separated by two-step unidimensional development so that their total quantities were presented in all examination results. By the application of two-dimensional chromatography, only traces of lysophosphatidylcholine were established in fresh meat. Phospholipids, namely isolated spots marked with X_s, X_1, X_2 and X_3 were not identified.

There are few data on the quantities of individual PL and they are mainly presented as percentages in total PL. Comparison of our results with the findings of Wood and Lister (27) and Arroyo and Aberle (1), who have isolated as yet the highest number of PL just in M. longissimus dorsi of hogs, showed that our results were more in concordance with the results of Wood and Lister (27). In our examinations, however, there were isolated phosphatidic acid, glicerophosphatidylcholine and four unidentified components, the quantities of which are not stated in the available literature, at least when red meat is in question.

The examination results of the effect of mechanical and heat treatments on the PL content and on oxidation and hydrolysis of lipids are presented in tables 2 and 3.

Calculated on dry matter basis, 98,28% of PL was established in the homogenate and 99.13% in meat pieces, in relation to their initial quantities in fresh meat. Grinding causes higher rate of hydrolysis and oxidation of lipids, first of all PL. Taking into consideration almost the same values for pH of meat, water content and sodiumchloride content, differences in the rate of oxidation and hydrolysis between lipids of homogenate and lipids of meat pieces can be ascribed to the influence of tissue desintegration. Considerably higher PV and TBA in raw homogenate than in meat pieces are in our opinion due to the exposure of a larger surface to the activity of oxygen and the increased possibility of oxygen incorporation into meat during homogenate preparation.

In relation to pasteurization, sterilization causes higher decrease of PL content and higher increase of FFA, both in homogenate and in meat pieces. The obtained results agree with the findings of Korzeniowski (15) stating that higher temperatures of heat treatment cause higher decrease of PL content. Contrary to that, higher oxidation was registered in pasteurized homogenates and pasteurized meat pieces in relation to sterilized samples. The obtained results

Changes of phospolipid content dependent on the way of mechanical and heat treatments

Table 2

Treatment	$\bar{x}$	Decrease of phospholipid content in % on wet weight	Decrease of phospholipid content in % on dry matter	Differences**	$\lvert d\bar{x} \rvert$	$\lvert d\bar{x}_{\%M/DM} \rvert$
Mechanical treatment				M:a	0.033 ···	1.71 ··
				M:b	0.028 ··	0.86 ·
a. homogenate	0.552	-	-	a:b	0.005	0.85 ·
M-a/M	-	5.64	1.72	$a:a_1$	0.022 ···	4.00 ···
b. meat pieces	0.557	-	-	$b:b_1$	0.014 ·	2.16 ··
M-b/M	-	4.78	0.87	$a_1:b_1$	0.013 ··	2.69 ··
Heat treatment				$a:a_2$	0.028 ···	4.74 ···
1. Pasteurization				$b:b_2$	0.021 ···	3.48 ···
a_1. homogenate	0.530	-	-	$a_2:b_2$	0.012 ··	2.11 ··
$a-a_1/a$	-	3.98	4.04	$a_1:a_2$	0.006	0.74
b_1. meat pieces	0.543	-	-	$b_1:b_2$	0.007	1.32
$b-b_1/b$	-	2.59	2.16	M:c_1	0.013 ··	13.11 ···
2. Sterilization				M:c_2	0.025 ···	7.33 ···
a_2. homogenate	0.524	-	-	$b_1:c_1$	0.041 ···	12.25 ···
$a-a_2/a$	-	5.07	4.80	b:c_2	0.053 ···	6.47 ···
b_2. meat pieces	0.536	-	-	$c_1:c_2$	0.012 ··	5.78 ···
$b-b_2/b$	-	3.77	3.52	$c_1:b_1$	0.055 ···	10.09 ···
3. Roasting				$c_1:b_2$	0.062 ···	8.77 ···
c_1. surface layer	0.598	-	-	$c_2:b_1$	0.067 ···	4.31 ···
$M-c_1/M$	-	2.22*	13.14	$c_2:b_2$	0.074 ···	2.99 ···
$b-c_1/b$	-	7.36*	12.37			
c_2. central part	0.610	-	-			
$M-c_2/M$	-	4.27*	7.33			
$b-c_2/b$	-	9.51*	6.52			

$\bar{x}$ - mean value of phospholipid content, %
M - fresh meat
* - "increase" of phospholipid content
** - $\lvert d\bar{x} \rvert$ - abosolute value of the difference of phospholipid content
$\lvert d\bar{x}_{\%M/DM} \rvert$ - absolute value of the difference of phospholipid percentage in sample in relation to its quantity in fresh meat (M), expessed on dry matter basis (DM)

Changes of peroxide value[1], TBA number[2] and free fatty acids[3] dependent on the way of mechanical and heat treatments[4]

Table 3

Differences Treatments	PV	TBA	FFA
a:b	1.68 ···	1.95 ···	0.84 ·
$a_1:b_1$	1.75 ···	1.37 ···	0.99 ··
$a_2:b_2$	1.39 ···	1.33 ···	0.95 ·
$a_1:a_2$	0.53 ·	0.57	0.20
$b_1:b_2$	0.17	0.53 ··	0.24
$a_1:a$	1.49 ···	0.99 ·	0.92 ··
$a_2:a$	0.96 ··	0.42	1.12 ·
$b_1:b$	1.42 ···	1.57 ···	0.77 ·
$b_2:b$	1.25 ··	1.04 ···	1.01 ··
$c_1:b$	4.77 ···	4.46 ···	1.99 ··
$c_2:b$	3.68 ···	3.24 ···	1.48 ··
$c_1:c_2$	1.09	1.22	0.51
$c_1:b_1$	3.35 ···	2.89 ···	1.22 ··
$c_1:b_2$	3.52 ···	3.42 ···	0.98 ··
$c_2:b_1$	2.26 ···	1.67 ···	0.71
$c_2:b_2$	2.43 ··	2.20 ···	0.47

1) meq peroxide/1000 g of lipids
2) mg malonaldehyde/1000 g of lipids
3) % oleic acid in 100 g of lipids
4) letters a, b, a_1, b_1, a_2, b_2, c_1 and c_2 denote the same mechanical and heat treatments as in table 2

$\lvert d\bar{x} \rvert$ - absolute value of mean values difference
$\cdot P < 0.05$ $\cdot\cdot P < 0.01$ $\cdot\cdot\cdot P < 0.001$

are in concordance with the statement of Yamauchi (29) that higher TBA are registered in pork when lower temperatures of heat treatment are applied. The decrease of PL content and the increase of FFA, PV and TBA were more markedly expressed in pasteurized, namely sterilized homogenates than in meat pieces heat treated in the same way. The established differences were significant in all cases.

Roasting causes considerably higher changes than pasteurization and sterilization. First of all, the PL content is considerably decreased by roasting, especially in the surface layer. In roasted meat, the decreased water content should particularly be kept in mind; otherwise a wrong impression of the "increase" of PL content would be obtained. Immediately after roasting and cooling, the PL content in the surface layer of meat pieces was 86.90% and in the central part - 92.58%, in relation to their quantities in fresh meat. The increase of FFA was also higher in the surface layer. If sterilized and pasteurized meat pieces as well as sterilized and pasteurized homogenates are considered comparatively, it can be concluded that roasting causes higher decrease of PL in the surface layer than mechanical treatment and pasteurization, namely sterilization, together. Higher increases of PL and TBA were registered in roasted meat in relation to pasteurized and sterilized homogenates, the increase being considerably higher in relation to pasteurized and sterilized meat pieces. This is probably the consequence of different activity rate of oxygen which was more pronounced during roasting due to the presence of more oxygen, whereas during sterilization and pasteurization less oxygen was available in unvacuumed cans. It is also possible that pronounced oxidation of pigments in roasted meat accelerates the lipid oxidation (6,9). Differences in PV, TBA and FFA between the surface layer and the central part were not significant.

On the basis of the established contents of PL, neutral and total lipids as well as PV, TBA and FFA determined in them, the data on the contribution percentage of PL and neutral lipids in oxidation and hydrolysis of lipids were obtained (table 4). Means and variance coeffi-

cients indicate the importance of PL contribution to the lipid oxidation and hydrolysis in fresh meat and in heat treated meat. The established contribution of PL in PV was 83-88% and in the TBA - 91-94%. High contribution of PL in PV and TBA, in relation to neutral lipids, can be explained by their liability to oxidation due to chemical composition. The contribution of PL in FFA ranged from 65 to 68%. This finding confirms the statement of Davidkova and Khan (5) that about 70% of the total increase of FFA in turkey meat is due to the effect of phospholipase B, and the rest of 30% to triacylglicerol decomposition.

Our further examinations will include examination of the analysed changes of lipids, namely PL, during different ways of storage as well as examination of the effect of different ways of mechanical and heat treatments and storage on the changes of contents of individual PL.

Contribution of phospholipids (%) in lipid oxidation and hydrolysis

Table 4

Samples		Peroxide value	% in TBA number	FFA
a. Homogenat				
raw	$\bar{x}$	86.25	91.45	65.41
	Cv	3.43	3.45	7.49
pasteurized	$\bar{x}$	86.38	93.11	65.11
	Cv	6.42	3.16	7.20
sterilized	$\bar{x}$	85.13	92.30	66.26
	Cv	6.10	2.85	13.17
b. Meat pieces				
raw	$\bar{x}$	83.12	90.98	64.98
	Cv	5.92	3.57	7.15
pasteurized	$\bar{x}$	83.23	92.70	65.11
	Cv	10.45	3.18	11.20
sterilized	$\bar{x}$	82.14	92.42	65.46
	Cv	11.42	3.84	7.53
c. Roasted meat				
surface layer	$\bar{x}$	88.22	94.65	67.60
	Cv	6.55	3.76	15.45
central part	$\bar{x}$	88.22	93.81	66.31
	Cv	6.65	3.65	9.31

References:

1. Arroyo P.T., Aberle E.D. 1970. J. Animal Sci. 31, 180* 2. Bunn C.R., Keele B.B., Elkan G.H., 1969. J. Chromatog. 45, 326* 3. Campbell A. M., Turkki P.R. 1967. J. Food Sci. 32, 143* 4. Carroll K.K. 1963. J. Am. Oil Chemist'Soc. 40, 113* 5. Davidkova E. Khan A.W. 1967. J. Food Sci. 32,35* 6. Eriksson C.E., Olsson P.A. Svensson S.G. 1971. J. Am. Oil Chemists'Soc. 48,44 7. Folch J., Lees M., Sloane Stanley G.H. 1957. J. Biol. Chem. 226, 497* 8. Grigor M. R., Moehl A., Snyder F. 1972. Lipids 7, 766* 9. Hirano Y., Olcott H.S. 1971. J. Am. Oil Chemists'Soc. 48, 523* 10. Jakubov G.Z. et. al. 1975. 21st Europ. Meeting of Meat Res. Workers' Berne* 11. Keller J.D., Kinsella J.E. 1973. J. Food Sci. 38, 1200* 12. Keljman L.F., Ljaskovskaja J.N. 1971. 18th Europ. Meeting of Meat Res. Workers', Bristol, E3* 13. Keljman L. 1967. Mjasnaja industrija SSSR, 2, 31* 14. Keskinel A., Ayres J.C., Snyder H.E. 1964. Food Technol. 2, 101* 15. Korzeniowski W. 1971. Roczniki Technologii i Chemii Zywnosci 21, 79* 16. Krzywicki K., Ratcliff P.W. 1967. J. Sci. Food Agr. 18, 252* 17. Nakanishi T., Suyama K. 1967. Nippon Chikusan Gakkai-Ho 38, 481. Summarized in: 1968. Fleischwirtschaft, 12, 1672* 18. Nešković N.M., Kostić D.M. 1968. J. Chromatog. 35, 297* 19. Oštrić-Matijašević B. 1962. Doctor thesis, Beograd* 20. Šmidt E.T., Ljaskovskaja J.N. 1973. Voprosi pitanija 4, 56* 21. Snedecor G.W., Cochran W.G. 1967. Statistical Methods, Yowa State University Press Ames, Yowa, U.S.A.* 22. Švadronova S.G., Vasiljeva N.G., Jakubov G.Z. 1977. 23rd Europ. Meeting of Meat Res. Workers, Moskva* 23. Tarladgis B.G., Watts B.M., Younathan M.T., 1960. J. Am. Oil. Chemists' Soc. 37, 44* 24. Turkki P.R., Campbell A.M. 1967. J. Food Sci. 32, 151* 25. Turner W.E., Paynter D.W., Montie J.E., Bessert W.M., Struck M.G., Olson C.F. 1954. Food Technol. 7, 326* 26. Wilson B.R., Pearson A.M., Shorland F.B. 1976. J. Agr. Food Chem. 24, 7* 27. Wood J.D., Lister D. 1973. J. Sci. Food Agr. 24, 1449* 28. Wuthier R.E. 1966. J. Lipid Res. 7, 544 * 29. Yamauchi K. 1972. Miyazaki Diagaku Nogakubu Kenkye Jiho 19, 1, 143. Summarized in: 1973. Food Sci. Technol. Abstr. 5,9, 168* 30. — 1965. Nordisk Metodik-Komite for Levnedsmidler, Nr. 57.