INVESTIGATIONS OF LIPID-PROTEIN INTERACTIONS IN MODEL SYSTEMS

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INTRODUCTION

During storage of foods numerous chemical processes - oxydation, hydrolisis, enzyme reactions - can proceed, which cause the deterioration of food products.

When storing foods of high fat content, the oxydative deterioration may be one of the most typical processes. The unsaturated fatty acids can be oxidized easily and a decrease of the organoleptic quality may be observed as a consequence of this oxidation. Recently it has become evident that lipid-protein complexes may be formed in addition to oxidation of unsaturated fatty acids. These complex compounds may cause radical changes in the foods, especially in the meat and fish products.

The mechanism of the reaction, taking place between proteins and lipids are not well understood. According to our present knowledge the following are the main reactions:

a./Formation of complexes; in which the hydrogen bonds are formed between lipids and proteins /Narayan et al /1964/

b./Formation of protein radicals/Roubal/1970/

c./Formation of protein - protein crosslinks. Protein - protein crosslinks can form in the presence of peroxidizing lipids, especially in that case, if the product has a high water activity /Kanner and Karez /1976/

d./Formation of protein - lipid crosslinks: some lipids and oxidizing lipids can bind covalently to proteins, forming lipid - protein crosslinks /Roubal and Tappel /1966/

One of the greatest problems is the formation of crosslinks, especially the formation of lipid - protein crosslinks during storage of meat products.

Andrews et al. /1965/ and Shin et al. /1972/ have described the formation of inter- and intramolecular crosslinks between proteins and lipids in the presence of peroxidizing lipids.

The products of secondary oxidation of the unsaturated fatty acids e.g. aldehydes, ketones, epoxydes are mainly responsible for the formation of these crosslinks /Gardner /1975/. The role and importance of malonaldehyde in the formation of lipid-protein interactions has been elucidated /Kwon et al. /1965/. Malonaldehyde as a secondary oxidation product of fatty acids originates from the autoxidation of polyunsaturated fatty acids, containing two or more double bond and / or prostaglandin-like endoperoxydes /Pryor et al./1976/. Because of its bifunctionality formation of crosslinks between free amino groups of proteins is possible /Crawford et al. /1967/, Buttkus /1967/. The mechanism of the reactions between malonaldehyde and free amino groups has been described by Chio and Tappel /1969/. The reaction products of malonaldehyde and amino groups were defined as a conjugated Schiff-base, which is a yellow or colourless flourochrome /Chio and Tappel /1969/b/.

N-terminal amino acids, as well as lysine, methionine, and arginine play also an important role in the formation of lipid-protein crosslinks /Svedlanka /1975/.

The formation of lipid-protein crosslinks may cause a considerable decrease of the quality of meat products. As a result of these reactions the meat products can be discoloured, this is the so called non-enzymic browning /Porkorny et al. /1973/. Furthermore the formation of crosslinks lead to the decrease of water binding capacity, solubility, lysine, arginine, and methionine availibility /Kusi et al. /1975/, Chio and Tappel /1969/b/. As a consequence, the decrease of the nutritive value of the meat products can be observed, due to the decrease of the protein efficiency ratio, and net protein utilization /Obanu et al. /1976/.

In the present study the chemical and physical parameters influencing the formation of lipid-protein crosslinks have been studied in model systems.

MATERIALS

Trioleate: glycerol trioleate /BDH Chemical Ltd./

Ethanolamine /E/: 1,5 g of ethanolamine /Merck/ was dissolved in 10 ml ethanol, and mixed with 50gr trioleate. Ethanol was evaporated in vacuum.

Lysine /L/: 1 g of lysine /Merck/ was dissolved in 20 ml distilled water and mixed with 40 g completely fatless connective tissue, prepared from adipose tissues of pig. The material was dried by air.

Peroxydes /PO/: 3,2 g of ciklohexanol peroxyde /Merck/ was dissolved in 20 ml ethanol and mixed with 50 g trioleate. The solvent was evaporated in vacuum.

Malonaldehyde /MA/: 1,5 g tetraethoxy propane /malonaldehyde-acethal/ was dissolved in 25 ml acetone and mixed with 20 g of DOWEX 50 x 4 /400 mesh/ resin, which was prepared in acidic form. The mixture was stored over 24 hours at 4°C. The malonaldehyde was dissolved in 20 ml acetone. The malonaldehyde solution was mixed with 50 g trioleate and the solvent was evaporated in a vacuum.

Tocopherol: acetate /Merck/

Composition of model systems: The model systems consisted of trioleate and the mixtures prepared as described above. The composition of the model systems are shown in Table 1.

METHODS

Determination of discoloured compounds - The amount of discoloured polymers derived from reaction between lipid oxiation products and amino acids were determined by Pokorny /Pokorny et al. /1973/, on the basis of the absorbance at 400 nm. The determinations were carried out in chloroformic solution. The absorbance was measured with Spectronom 204 spectrophotometer.

Determination of fluorochromes - The fluorecence intensity of lipid-protein crosslinks was determined by method of Fletcher /Fletcher et al. /1972/. The wavelength of the excitation was 366 nm, and emmission was measured at 460 nm, with spectrofluorometer EEL 244 /Evans Ltd/.

RESULTS

The formation of lipid-protein crosslinks was studied in model systems in order to prove the effects of the different kinds of lipid oxidation products, and amino-compounds on the formation of coloured polymers and fluorochromes, which have caused the quality deterioration of the meat products during storage.

The influence of primary/peroxyde/ and secondary /malonaldehyde/oxidation products were estimated among the products of oxidizing fatty acids. Ethanolamine as a basic component of phospholipids and lysine were studied among the amino-compounds.

Furthermore we have studied the influence of the homogenity of the mixtures, and the keeping quality /temperature and time/. /The mixture containing ethanoamine was homogenous and the one containing lysine was heterogenous./

The models have been studied at 6°C, according to the industrial practice, and at room temperature /25°C/ for ten weeks. At both temperatures controls were as follows: trioleate /T/, trioleate and PO /K_{PO}/, trioleate and MA /K_{MA}/, trioleate and ethanolamine /K_E/ and trioleate and L /K_L/. The concentrations of oxidation products and amino-compounds were the same both in controls and models. During ten weeks storage the amount of coloured polymers and the intensity of flourochromes were measured every three days. The results are shown in Tables 2-5.

On the basis of the results it has been found that each of the models discoloured and the flourescence intensity has been increased during storage. The controls have not been discoloured during this time, except the sample K_E, which contained ethanolamine.

The results were evaluated by analysis of variance. The mathematical analysis was carried out on the basis of factorials shown in Table 6. The program employed for the mathematical evaluation is suitable for performance of complete factorial analysis. In this case the program has been applicable for the estimation on data of the control, standard deviation, and for the analysis of the types of distribution. The results are summarized in Table 7.

DISCUSSION

The model systems, consisted of the oxidation products of lipids and amino-compounds have been studied in order to establish the influence of some chemical and physical parameters of the formation of lipid-protein crosslinks.

Chemical Composition

The influence of the oxidation products of lipids - On the basis of the Figures it can be seen that the amount of coloured polymers, characterized by the absorbance at 400 nm intensively increases in the presence of the peroxydes and malonaldehyde during storage. The increase is significant at both temperatures /Table 3./. The rate of increase is the highest during the first three weeks.

The increase of the amounts of coloured compounds is less intensive in the presence of PO, than in the presence of MA. The amounts of coloured polymers were increased by 241 p.c. /EPO/ and 48 p.c. /LPO/ in the presence of peroxyde, while the rate of increase was 356 p.c. and 59.6 p.c. /LMA/ in the presence of malonaldehyde.

The fluorescence intensity /the amount of fluorochromes/ has been changed in the same way as the absorbance, but the degree of increase is not so high. The fluorescence increased by 184 p.c. /EPO/ and 2.6 p.c. /LPO/ in the case of peroxyde. The fluorescence intensity of model systems, containing malonaldehyde has increased in similar way in the case of both amino-compounds. The degree of increase was 141 p.c. /EMA/ and 131 /LMA/.

On the basis of the difference between the increase of fluorescence and absorbance, it can be stated that some coloured compounds have been formed which were not fluorescent in the reaction of lipids and aminogroups.

The amino-compounds - Of the two estimated amino-compounds the ethanolamine has been shown higher activity. The increase of the amount of both coloured compounds and fluorochromes were more intensive. Furthermore the control containing ethanolamine /K_E/ has shown a significant increase in absorbance at 400 nm, and in fluorescence at 460 nm. It is very interesting that only the amount of fluorochromes increased in the presence of lysine. The absorbance of coloured compounds also increased but the rate of increase was not significant.

It is presumable that the estimated difference between activity of ethanolamine and lysine may cause that the model, containing lysine was heterogenous and some of the coloured polymers could be associated with connective tissues.

The antioxidants - The effect of the antioxidant on the formation of lipid-protein crosslinks was studied by the tocopherol-acetate. Tocopherol as a natural antioxidant may be present in all of the meat products.

On the basis of these results it has been found that the formation of lipid-protein crosslinks did not depend on the amount or presence of tocopherols.

Physical parameters

Temperature - According to results of model experiments it has been found that the temperature may influence the formation of lipid-protein crosslinks to a small extent. A moderate increase of the absorbance could be observed due to the increase of temperature where the increase is significant, but the degree of the increase was very small. The increase of storage temperature did not cause a significant increase in the fluorescence intensity.

Time - The amount of coloured and fluorescent compounds significantly increased in function of time. The curves, time versus absorbance and fluorescence, are not linear. The most intensive increases of the amounts of coloured and fluorescent compounds can be observed in the first three weeks.

Summarizing the results it has been found that the reaction between lipids, their oxidation products and amino-compounds may be proceeded either at a lower or at a higher temperature, either in homogenous or in heterogenous phases. As a result of these reactions, yellow or brown polymers can form, some of which compounds may have fluorescence properties. The amounts of these compounds mainly depend on the presence of the oxidation products of unsaturated fatty acids, especially the presence of malonaldehyde and amino-compounds. The formation of lipid-protein crosslinks can not be prevented by antioxidants if the oxidation products of lipids have already been present. The changes of temperature influence the formation of lipid-protein crosslinks to a small extent. The increase of the amounts of coloured compounds is the most intensive in the first three weeks during storage.

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Table 1.: Composition of the model systems

Code	Trioleate /gr/	L	MA	E	PO	T
LMA	13,8	0,7	1,2	-	-	-
LMAT	12,6	0,7	1,2	-	-	1,2
LPO	13,0	0,7	-	-	2,0	-
LPOT	11,8	0,7	-	-	2,0	1,2
EMA	12,6	-	1,2	1,2	-	-
EMAT	11,4	-	1,2	1,2	-	1,2
EPO	11,8	-	-	1,2	2,0	-
EPOT	10,6	-	-	1,2	2,0	1,2

Table 2.

Factorials and factorial-levels

Factorial	Code	Number of factorial-levels	Characterization of factorial - levels
Temperature	1	2	1. 6°C 2. 25°C
Time	2	7	1. 0.week 2. 2.week 3. 3.week 4. 4.week 5. 5.week 6. 6.week 7. 7.week
Oxidation products	3	3	1. without oxidation products 2. in the presence of PO 3. in the presence of MA
Amino-compounds	4	2	1. withouth amino-compounds 2. in the presence of E or L

Table 3.: Results of analysis of variance

	Significant factorials		Second and third significant interactions		Increase in percent	
	E^x	L^{xx}	E	L	E	L
Absorbance at 400 nm	Temperature/1/ Time/2/ Oxidation products/3/ Ethalonime/4/	Temperature /1/ Time/2/ Oxidation products/3/	14; 124; 24; 134;	23; 124; 14; 134; 24; 214; 34;	Temperature: 9,4 PO*:241,0 Ma**:355,0 E: 901,1	Temperature: 8,9 PO:48,80 Ma: 59,60
Fluorescence at 460 nm	Time/2/ Oxidation products/3/ Ethanolamine /4/	Time/2/ Oxidation products/3/ Lysine/4/	13; 124; 23; 134; 24; 214;	23; 124;	PO: 184,0 MA: 141,5	PO: 2,6 MA: 131,40

^{x}E: model with ethanolamine

^{xx}L: model with lysine

*PO: model with ciklohexand peroxyde

**MA: model with malonaldehyde

Table 2.: Results of model experience at 25°C

SAMPLE	TIME /week/	Absorbance at 400 nm								Fluorescence at 400 nm							
		0	1	2	3	4	5	6	10	0	1	2	3	4	5	6	10
LMA		0,07	1,0	0,95	1,2	0,8	0,85	0,90	0,95	40	44	45	50	47	77	95	120
LMAT		0,07	1,0	1,03	1,0	0,8	0,8	0,90	0,95	40	50	48	48	51	70	90	100
LPO		0,07	0,5	0,6	0,7	0,6	0,6	0,6	0,65	30	35	33	33	34	35	35	36
LPOT		0,07	0,5	0,7	0,8	0,65	0,6	0,65	0,65	30	29	32	33	32	34	34	34
EMA^x		0,07	4,1	4,2	4,0	6,2	6,6	6,8	6,8	38	192	800	900	990	700	800	900
EMAT^x		0,07	4,0	4,4	4,4	6,3	6,2	6,2	6,2	40	194	450	900	1000	688	780	760
EPO^x		0,07	1,35	4,4	5,2	4,2	4,6	4,6	4,8	73	705	620	720	740	720	720	780
EPOT^x		0,07	1,30	4,6	5,8	4,6	4,8	4,8	4,8	73	750	720	900	940	880	880	890

^{x} accounted values

Table 3.: Results of model experience at 6°C

SAMPLE	TIME /week/	Absorbance at 400 nm								Fluorence 460 nm							
		0	1	2	3	4	5	6	10	0	1	2	3	4	5	6	10
LMA		0,07	0,8	1,0	1,2	1,1	1,1	1,0	1,0	40	73	74	71	92	90	90	100
LMAT		0,07	0,8	1,0	1,1	1,0	1,1	1,1	1,1	40	71	75	72	94	96	100	110
LPO		0,07	0,7	0,75	0,8	0,85	0,8	0,9	0,95	30	35	33	33	34	34	41	45
LPOT		0,07	0,6	0,65	0,92	0,75	0,8	0,85	0,85	30	34	37	34	34	35	35	40
EMA^x		0,07	4,1	5,6	6,6	5,6	5,2	5,7	5,8	38	192	420	526	528	500	640	840
EMAT^x		0,07	4,0	4,6	4,6	4,6	4,9	4,9	5,0	39	180	380	400	440	448	680	800
EPO^x		0,07	1,35	3,1	5,3	4,6	4,8	5,0	5,2	73	340	672	600	600	700	780	700
EPOT^x		0,07	1,3	4,6	5,8	4,0	3,8	4,0	4,8	73	425	712	720	740	700	725	700

^{x} accounted values

Table 4.: Results of controls at 25°C

CONTROLS	TIME /week/	Absorbance at 400 nm								Fluorescence at 460 nm							
		0	1	2	3	4	5	6	10	0	1	2	3	4	5	6	10
K		0,04	0,05	0,06	0,06	0,06	0,06	0,06	0,06	27	32	28	29	33	37	38	39
K_T		0,04	0,05	0,07	0,08	0,06	0,06	0,06	0,06	28	33	31	28	35	32	33	34
K_K		0,04	0,07	0,05	0,08	0,07	0,07	0,07	0,07	28	28	29	34	31	32	32	35
K_P		0,045	0,05	0,06	0,055	0,06	0,065	0,06	0,065	28	31	30	30	32	35	35	38
K_E		0,10	0,09	0,11	0,19	0,13	0,18	0,18	0,19	29	29	30	31	31	31	31	32
K_{MA}		0,07	0,07	0,08	0,09	0,10	0,09	0,08	0,09	30	35	40	45	50	45	45	42

Table 5.: Results of controls at 6°C

CONTROLS	TIME /week/	Absorbance at 400 nm								Fluorescence at 460 nm							
		0	1	2	3	4	5	6	10	0	1	2	3	4	5	6	10
K		0,04	0,07	0,06	0,06	0,06	0,06	0,06	0,065	27	28	29	34	31	32	34	35
K_T		0,04	0,06	0,07	0,06	0,05	0,05	0,06	0,07	28	28	32	32	31	32	32	32
K_K		0,04	0,07	0,07	0,07	0,06	0,05	0,06	0,06	28	32	28	28	31	32	33	35
K_P		0,045	0,06	0,05	0,06	0,06	0,05	0,05	0,05	28	29	29	33	31	32	32	35
K_E		0,04	0,10	0,09	0,09	0,12	0,11	0,10	0,10	29	29	29	33	31	31	33	33
K_M		0,07	0,09	0,09	0,09	0,10	0,10	0,10	0,10	35	39	43	50	50	49	48	50