STUDIEN ÜBER DIE VERFARBUNG DES SPECKES

1. VORKOMMEN DER LIPOFUSCINARTIGEN FARBSTOFFEN

Á.JUHÁSZ, É.BERNDORFER-KRASZNER, L.KÖRMENDY and T.GÁBOR

Ungarisches Forschungsinstitut für Fleischwirtschaft und Institut für Biochemie und Lebensmitteltechnologie der Technischen Universität, Budapest

Zusammenfassung

In den letzten Jahren wurde in Ungarn festgestellt dass die gesalzen gelagerten Rückenspecke der Schweine von Fall zu Fall eine gelbe Verfärbung zeigten. Diese Veränderung verursacht bedeutende Schwierigkeiten bei der weiteren Verarbeitung. Die Verfasser haben das Mechanizmus der Verfärbung der Rückenspecke, weiter die chemische Eigenschaften der entstandenen Farbstoffen, und die Trennungsmöglichkeiten umtersucht.

Weiter wurden die im Rückenspeck entwickelte gelbe Pigmente säulenkromatografisch auf drei verschiedenen Fraktionen /K, M und E/ getrennt. Sie untersuchten auch die UV, IR und fluorescens Spektren des Farbstoffes. Es wurde festgestellt, dass alle drei Fraktionen lipofuscinartige Pigmente sind. Es ist weiter anzunehmen, dass die Fraktionen in folgender Reaktion entstehen: in der Oxidation der Fetten entstandene sekunder Produkten z. B. Malonaldehyd reagiert mit der Amin- Gruppen der Proteinen und Phospholipiden wobei gelbe Schiff - Basen entstehen.

Es ist noch unbekannt welche biologische und biochemische Prozesse bei der Bildung der gelben Pigment eine Rolle spielen. Zur Elklärung dieser Fragen wünschen die Autoren weitere Untersuchungen durchführen. Sie setzen voraus, dass zwei Faktoren: die Mangel der F Vitamine und die Stress- Wirkung eine Bedeutung hätten.

ÉTUDES SUR LA DÉCOLORATION DU LARD PORCIN

1. PRÉSENCE DES PIGMENTS DE TYPE DE LIPOFUSCINE

Á.JUHÁSZ, É.BERNDORFER-KRASZNER, L.KÖRMENDY and T.GÁBOR

Institut de Recherche sur les Viandes, Budapest et Institut de Biochimie et de la Technologie des Produits Alimentaires, Université des Sciences Techniques, Budapest

Résumé

Dans les dernières années, on constata en Hongrie, que le tissu adipeux du porc jaunissait parfois après une salaison de 14-20 jours. Les auteurs examinèrent le méchanisme de la coloration jaune des lards c.à.d. la composition chimique des matières colorées et la possibilité de leur séparation.

Ils examinèrent la corrélation entre la teneur en vitamine E et en acides gras insaturés du tissu et ils constatèrent que le phenomène du jaunissement n'est pas en relation étroite avec la teneur en vitamine E ou avec la quantité des acides gras insaturés.

Dans la suite, il séparerent les pigments jaunes à trois fractions par une méthode chromatographique. Ils examinerent aussi les spectres UV, IR et de fluorescence de ces fractions et constatèrent que tous les trois fractions colorantes sont analogues aux pigments de lipofuscine. Probablement les produits secondaires de l'oxidation des matières grasses réagissent avec les groupes d'amine primaires des protéines ou avec les phospholipides formant des produits jaunes de type de base Schiff.

Les résultats des analyses n'éclairent pas les effets biochimiques et biologiques causant la formation des lipofuscines. Les auteurs supposent que les facteurs responsables sont la manque de la vitamine E et l'action du stress.

STUDIES ON THE DISCOLORATION OF ADIPOSE TISSUE OF PIGS

1. OCCURENCE OF LIPOFUSCIN-LIKE PIGMENTS

Á.JUHÁSZ, É.BERNDORFER-KRASZNER, L.KÖRMENDY and T.GÁBOR

Hungarian Meat Research Institute and Institute of Food Biochemistry and Technology University of Technical Sciences, Budapest

Summary

In Hungary it was observed that the dry cured back fat of pig turned sometimes yellow within 14-20 days giving rise to considerable difficulties in the further processing. The mechanism of discolouration, the chemical characteristics of the pigments and the possibility of their separation were studied.

The yellow pigments of back fat were separated to 3 different factions /K, M and E/ by column chromatography. The UV, IR and fluorescence spectra of the fractions were lipofuscin-like pigments and possibly they were formed in the following chemical process: the secondary products of fatty acids oxidation, e.g. malonaldehyd, linked with the primer amino groups of proteins or phospholipids produce yellow Schiff- base type. compounds. Physiological or biochemical effects producing the lipofuscin-like pigments were not investigated yet. It is supposed that two factors could be important: the deficiency of vitamine E and stress.

ИЗУЧЕНИЕ ИЗМЕНЕНИЯ ЦВЕТА В ЖИРОВЫХ ТКАНЯХ СВИНЕЙ.

I. Присутствие пигментов липофусцинного типа.

А.ЮГАС, Е.БЕРНДОРФЕР-КРАСНЕР, Л.КЕРМЕНДИ и Т.ГАБОР.

/ Исследовательский институт мясной промышленности, Будапешт. и Институт биохимии и технологии пищевой промышленности, Технический Университет, Будапешт./

Резюме.

В Венгрии в последние годы обнаружили, что спинное сало свиней, хранящееся в засоленном состоянии, в нескольких случаях желтело за 14-20 дней. Это изменение цвета причиняет значительный денежный ущерб промышленности.

Авторы исследовали механизм изменения цвета сала, химическую природу образующихся цветов и возможность разделения цветных фракций.

На основании контрольных исследований авторы установили, что окраску сала не вызывают ни ошибки в технологии, ни заболевания животных. Авторы установили связь между содержанием витамина Е и ненасыщенными жирными кислотами в жировых тканях. Они установили, что желтение вызывает не только недостаток витамина Е и большое количество ненасыщенных жирных кислот. В дальнейших исследованиях желтый цвет, образующийся в спинном сале, разделили на три фракции/ К, М, Е фракции/ при помощи метода колонной хроматографии.

Авторы исследовали УФ и ИК, а также флуоресцентные спектры этих веществ. Они установили, что эти три цветные фракции, которые вызывают окраску сала являются пигментами липофусцинового типа и предполагают, что эти фракции образуются в результате следующего химического процесса: вторичные продукты, которые образуются при окислении жира, например, малональдегид, реагируют с первичными аминно-группами протеинов или фосфолипидов, одновременно образуя продукты желтого цвета, которые относятся к типу Шифф оснований.

Эти результаты исследований еще не дают объяснения на то, какие биологические или биохимические факторы влияют на образование пигментов липофусцинного типа.

Авторы желают провести дальнейшие исследования для выяснения этих вопросов. Они предполагают, что среди катализирующих факторов двум можно придать большое значение: недостатку витамина Е и влияние стресса.

STUDIES ON THE DISCOLORATION OF ADIPOSE TISSUE OF PIGS

1. OCCURENCE OF LIPOFUSCIN-LIKE PIGMENTS

Á.JUHÁSZ,[xx] É.BERNDORFER-KRASZNER,[xx] L.KÖRMENDY[x] and T.GÁBOR

Yellow or brown discoloration of adipose tissues is a long-known problem. The phenomenon can generally be attributed to various causes: deficiency of natural antioxidants resulting in intensive oxidative rancidity: oxidative colour changes accompanied by pigment formation: other disorders in oxygen supply.

The oxidation processes taking place in the tissues, and particularly the autooxidation of fatty acids are especially accelerated as compared to their usual rate by using feed deficient in vitamin E. This process is often indicated by a yellow discoloration of the adipose tissues /1, 2/. When occurring in the presence of higher amounts of unsaturated fatty acids, the discoloration can reach such an extent that the adipose tissues turn brown /3/. In the yellowing of fats caused by vitamin E deficiency sulphur-containing amino acids and selenium complexes also play an important part. However, the composition and structure of the colouring agents is as yet unknown /4/.

As a result of vitamin E deficiency, not only the autooxidation rate of the fats will increase, but other changes will also take place in the organism. E.g. yellow or brown pigments will be deposited in adipose tissues and in the liver. These pigments are termed lipofuscins or ceroids /5, 6/. Lipofuscin has long since been known in medical practice as a by-product of cellular metabolism appearing in older age /"age-pigment"/. Its composition and structure has not, however, been elucidated /7/. Lipofuscin is also formed in younger cells when disorder in the oxygen supply of the organism occurs /8/. Deposition of lipofuscin has been observed in liver /9/, in heart /10/, in nervous tissues /11/, in kidneys /12/ and in adipose tissues /13/.

Lipofuscin was also found in adipose tissues /14/, when - as a result of a stress state owing to various causes - important changes took place in fat metabolism, e.g. free fatty acids have been mobilized /15, 16/. This discoloration is sometimes termed "fat stress phenomenon" /15/.

Samorojski et.al /17/ and Chio et.al /18/ described lipofuscin-like pigments, which - though very similar to lipofuscin in properties - differ from the latter in their formation process. Thus, e.g. peroxides formed in the autooxidation of unsaturated fats will rapidly decompose into compound containing carbonyl groups, e.g. malonic aldehyde. In turn, the latter are capable of reacting with the primary amino groups of proteins or phospholipids, yielding yellow or brown polymers of the Schiff base type /18/. Since the fluorescence spectra of these products are very similar to that of lipofuscin, the yellow polymers were termed lipofuscin-like pigments. Feeding with fodder deficient in vitamin E largely promotes the formation of lipofuscin-like pigments /19/.

Pokorny and Janicek /20/ prepared yellow or brown polymers in model systems consisting of lipids with different degrees of unsaturation and albumin. The formation process was identical with that of lipofuscin-like pigments. So the term lipofuscin should rather be considered a collective term for all fat-soluble pigments formed in various processes.

During the last few years, it was observed in Hungary that the processed adipose tissues of pig /back fat/, in the dry salted state, turn yellow in about 14 to 20 days. The extent of yellowing largely differs from batch to batch. The usual veterinary control of the animals did not indicate any pathological changes. An examination of rancidity characteristics demonstrated that the yellow discoloration is not simply due to oxidative rancidity. This is also confirmed by the fact that all other organoleptic characteristics of the yellow fat remained unchanged.

It was attempted to attribute the discoloration of the pig back fat to one of the causes discussed in the foregoings.

Experimental

Sampling

We chose 10 samples of back fat from animals whose breed, sex, age and fodder composition was known /Table 1/.

Storage

The samples were dry cured and stored at +2 °C. The amount of salt /NaCl/ was 10% on the weight of the sample.

Testing methods

Determination of vitamin E. The total percentage of tocopherol in the sample was determined spectrophotometrically, using the Emmerie-Engel reaction /21/.

Identification of lipofuscin. Lipofuscin was determined in the yellow samples by a histochemical method /12/.

Tests of yellow coloring agents

Thin-layer chromatography. The presence of coloured substances in the samples was demonstrated by the TLC method reported by Pokorny /23/.

Recovery and separation of the pigments was carried out on DEAE-Cellulose by coloumn chromatography /25/. From the chloroformic eluate, a yellow oil was obtained /fraction K/. Evaporation of the chloroform-methanol eluent yielded a yellowish-brown, viscous product /fraction M/, while a dark brown solid substance was obtained from the chloroform-acetic acid fraction /fraction E/.

UV spectrophotometry. UV absorption spectra of the coloured substances were measured using a MOM 201 spectrophotometer in chloroformic solution.

IR spectrometry. IR absorption spectra were measured between two KBr plates, using an UR-20 IR spectrometer.

Fluorescence spectra. Fluorescence spectra were taken in chloroformic solution, using a Fluorimeter EEL 224.

Results

In studies on yellow discoloration of pig fat, first the relationship between vitamin E content and fatty acid composition of the adipose tissues was determined /Table 2/. Results demonstrated that - although feeding was identical - important differences existed among the total tocopherol contents of individual samples. The percentages of unsaturated fatty acids also very. The large differences between linolic acid percentages are particularly striking. However, the results do not unequivocally confirm the assumption that yellowing of the samples is caused by their low vitamin E content and high unsaturated fatty acid content.

Next it was attempted to confirm the presence of lipofuscin pigments in the samples. Histological investigations demonstrated that color formation is caused by a moderate amount of fat-soluble yellowish-green pigment particles that are, however, not identical with either lipofuscin or other pigments known up to the present /24/.

The next approach aimed to explain yellowing of pig back fat by the formation of lipofuscin-like pigments. It was therefore attempted to demonstrate the presence of yellow polymers formed by the mechanism, presented in Fig.1., using TLC /20/.

These studies confirmed that the samples contained coloured substances formed in the polymerization process which resulted from the primary oxidation stage /Fig.2/.

As described in the experimental part, three different coloured substance fractions /fractions K, M and E/ were isolated from the discoloured samples by column chromatography.

Table 3 shows the nitrogen content of these fractions, using the micro-Kjeldahl method.

The nitrogen content of the fractions K and M are of the same order of magnitude as that of the polymers prepared by Pokorny /23/. In contrast, the fraction E contains a relatively high amount of nitrogen, so that it may be assumed that a higher amount of protein participates in its formation.

Subsequently the pigments were identified by means of their absorption and fluorescence spectra.

The UV spectra of the pigment fractions are presented in Fig.3. A very characteristic maximum is observable in the spectra of fractions K and M at 246 nm and 247 nm, resp. Another low-intensity absorption peak is present in the spectrum of fraction M at 310 nm. The UV absorption spectrum of fraction E shows three relatively low-intensity peaks at 247, 270 and 340 nm.

IR absorption spectra allow to state /Figs 4-6/ that the isolated pigments consist of moderate chain-length unsaturated fatty acid esters /-C-O-C- peaks appear at 1243-1248 cm^{-1}, the carbonyl peak at 1758 cm^{-1} and the unsaturated carbon bond peak at 3015 cm^{-1}/.

Presumably the fraction K consists chiefly of fatty acid esters, since no peaks corresponding to primary and secondary amino groups are present in its IR spectrum. Tertiary amino groups might be present in this fraction, but their absorption bands could not be identified in the IR spectrum.

Fractions M and E also contain -OH groups /signal at 3600-3400 cm^{-1}/ and =NH- and $-NH_2$ groups /signal at 3200-3400 cm^{-1}/. It may therefore be assumed that phospholipids and/or proteins participate in the formation of these fractions.

The fluorescence spectra taken at an exciting wave-length of 380 nm demonstate the presence of an intense maximum for fractions K and E and of a weaker maximum for fraction M /Fig. 7/. The maximum is located at 460 nm, 430 nm and 455 nm for fractions K, E and M, resp. This is in good agreement with the data already published /18, 19/, according to which maximum fluorescence for lipofuscin-like pigments is in the range of 450-470 nm at exciting wave-lengths of 360-390 nm. /The characteristic wave-length of the autofluorescence of lipofuscin is also 460 nm./

Discussion

The yellow discoloration of the dry cured back fat of pig is a great problem in industrial practice, and - in particular - is a source of important financial losses, since the discoloured fat cannot be used for consumption. However, no reference to a similar industrial occurence could be found in the available literature. This can partly be explained by the fact that the processing of the raw back fat in the dry salted state to prepare cured or smoked bacon at a later time is the usual process applied in Hungary. By the new separation method reported three different fractions of pigment /K, M and E/ could be isolated. The UV, IR and fluorescence spectra show that all three pigment consist of moderate chain-length unsaturated conjugated fatty acid esters. Fractions M and E presumably also contain -OH and nitrogen-containing groups which could be -NH-NH-, -NH-CH-, -N=N- or $-NH_2$ groups. However, the location and number of these groups, f.e. the exact structure of the pigment fractions cannot be determined on the basis of UV and IR spectra. The absorption bands characterizing these groups are not yet published.

Analysis of the spectra and TLC studies demonstrated that the pigments isolated from the tissues are identical or very similar to the lipofuscin-like pigments already published /18, 20/. Hence, these pigments are presumably formed by the chemi-

x Hungarian Meat Research Institute, Budapest

xx Institute of Food Biochemistry and Technology, University of Technical Sciences, Budapest

cal reactions presented in Fig.1. As a result, products of the Schiff base type, containing several chromophores, are formed of characteristic fluorescence maxima at 450-470 nm /with exciting wave-lengths of 360-390 nm/.

On the basis of the experimental results, it may be assumed that, among the pigments isolated from the yellow adipose tissues, fraction K is mainly the product of the reaction between malonic aldehyde and lipids. Phospholipides are probably reacting with malonic aldehyde to yield fraction M, while the reaction of proteins with malonic aldehyde appears to be responsible for the formation of fraction E.

However, these results do not statisfactorily explain why the formation of lipofuscin-like pigments takes place so rapidly, and why intense discoloration appears only with some of the samples. In fact, the conditions necessary for the reaction of pigment formation e.g. peroxides from unsaturated fatty acids, malonic aldehyde or some other compound containing a carbonyl group, phospholipides and proteins are present in all adipose tissues.

It therefore appears probable that the animals whose adipose tissue turns yellow during storage have been subjected to such environmental or biochemical impacts that start or catalyze pigment formation. Based on data already published and on the present experimental findings, major importance should be attributed in particular to two factors, viz. to the changes in vitamin E levels and to the stress effects provoked by environment.

References

1. Dam, H., J.Nutrit, 27 193 /1944/
2. Dam, H., Granados, H., Brit.J.Nutrit., 3 322 /1949/
3. Robinson, K.L., Coey, W.E., Nature, 997 /1951/
4. Takarmány ártalmak és hiánybetegségek. Mezőgazdasági Kiadó, Budapest 1972.
5. Noborn Nishioka, Seishin Shinkeigaku 68 836 /1966/
6. Wei King, D., Kusum Verma, J.Nutr., 91 159 /1967/
7. Seibert, Diesel, Jahr, Histochemie, 2 17 /1962/
8. Bjoerkerud, S., Cummius, J.T., Exptl.Cell.Res. 33 510 /1963/
9. Berneis, K.H., Studer, A., Pathol.Mikrobiol. 31 108 /1968/
10. Heidenreich, O. Virch.Arch.Pathol.Anal.Pysiol., 827 112 /1965/
11. Barka, T., Schener, P.J., Shaffner, F., Arch.Pathol. 78 331 /1964/
12. Harris, Ch., Arch.Pathol., 82 353 /1966/
13. Kenkichi Yokohama, Kazdho Ishida Tohoku, J.Agr.Res., 8 113 /1958/
14. Nikodijevic, O., Kovacev, S., Acta Med.Jugosl., 26 149 /1972/
15. Hayes, Nielsen, Rousseau, J.Nutr., 99 196 /1969/
16. Studer, A., Schaerer, K., Schweiz.Med.Wochsch. 95 933 /1965/
17. Samorojski, T., Keefe, J.R., J.Gerontol., 19 262 /1964/
18. Chio, K.S., Tappel, A., Reiss, U., Science, 166 1535 /1969/
19. Tappel, A., Reddy, K., Fletcher, B., J.Nutr. 103 908 /1973/
20. Pokorny, J., Janicek, G., Die Nahrung, 17 545 /1973/
21. Berndorfer-Kraszner, É., Dissertation, Budapest 1966
22. Krutsay, M., Morfol. Igazságügyi Orv.Szemle, 11 212 /1971/
23. Pokorny, J., Janicek, G., ZLUF, 151 31 /1973/
24. Józsa, L., Verbal communication /1973/.
25. Juhász, A., Berndorfer-Kraszner É., Kormendy, L. Acta Alimentaria /under press/.

Fig.1

Formation process of lipofuscin-like pigments

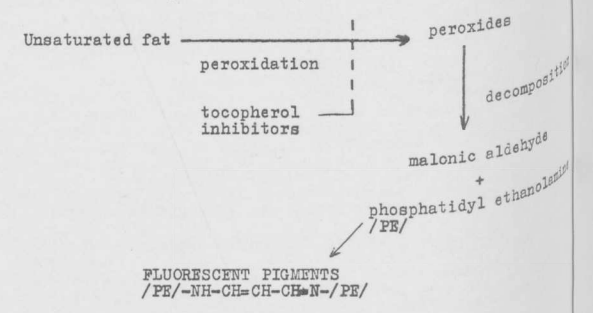

Table 1. Characteristic data of the investigated pig back fats

Sample No.	Breed	Sex	Age /days/
1	ULxH	♂	219
2	UxL	♂♀	228
3	UxL	♂♀	221
4	UxL	♂♀	228
5	ULxL	♂♀	215
6	UxL	♂♀	221
7	UxL	♂♀	219
8	ULxH	♀	234
9	ULxL	♀	215
10	ULxH	♀	223

U = Hungarian White L = Dutch Flatland
H = Hampshire

Table 1 a. Fodder composition

Maize %	Wheat %	Soya 50 %	Biolysine %	AP-14 %	Salt %	Fodder %	Premix % [x]
61	14,2	22,0	0,4	0,5	0,4	1,0	0,5

[x] According to Hungarian Standard

Table 2. Vitamin E content, fatty acid composition and discoloration data of the samples[x]

Trial	T_1 mg/100g	T_2	Yellowing	Total fat %	Ratio of unsaturated and saturated fatty acids	Distribution of fatty acids 14:0 /%/++	16:0 /%/	18:0 /%/	16:1 /%/	18:1 /%/	18:2 /%/	18:3 /%/
1	1,83	0,730	+	93,73	1:1,286	1,307	25,474	14,209	4,206	37,860	10,656	0,022
2	1,307	0,555	-	96,46	1:1,712	1,409	22,551	11,597	2,609	46,983	8,820	0,025
3	0,975	0,125	-	93,55	1:1,660	0,873	22,736	11,709	3,638	41,823	13,307	0,020
4	0,825	0,655	±	97,72	1:1,499	1,373	24,179	14,343	3,296	44,512	10,00	0,021
5	0,500	0,450	+	95,14	1:1,872	1,565	18,024	13,536	5,114	38,625	18,268	0,012
6	0,480	0,475	+	89,05	1:1,331	1,415	26,624	13,709	1,906	35,877	9,516	0,009
7	0,400	0,320	±	96,15	1:1,213	10,936	21,200	11,306	3,531	44,927	4,238	0,011
8	0,270	0,263	-	96,35	1:1,609	1,807	22,458	12,660	4,144	42,203	13,051	0,022
9	0,255	0,120	+	97,10	1:1,393	2,248	25,487	12,834	2,011	43,517	10,970	0,033
10	0,140	0,126	±	97,74	1:1,680	0,997	21,705	13,786	5,010	46,444	9,774	0,042

T_1 = total tocopherol content after slaughtering
T_2 = total tocopherol content after two weeks storage
x Data are mean values of three parallel determinations.
xx Percentages are related to the sample weight.

Table 3. Nitrogen content of yellow pigments formed at the oxidation of fats

Sample	Nitrogen content, %
according to Pokorny et al. /23/	0.4 - 1.0
Fraction K	0.60
Fraction M	0.82
Fraction E	2.80

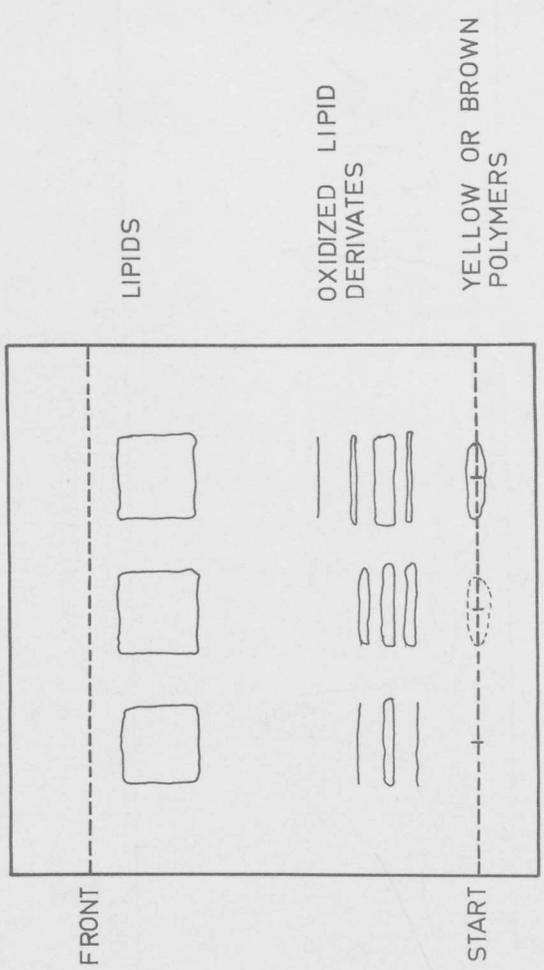

Fig. 2.

Detection of polymers by thin layer chromatography.

Solvent: benzene/ acetone/ acetic acid 95:5:1

Adsorbent: Kieselgel G

Spray reagent: a 2o % alcoholic solution of phospho-molibdic acid

A: a back fat sample 24 hours after slaughtering

B: a salted back fat sample after a 2 week storage

C : a yellow salted back fat sample after a 2 week storage

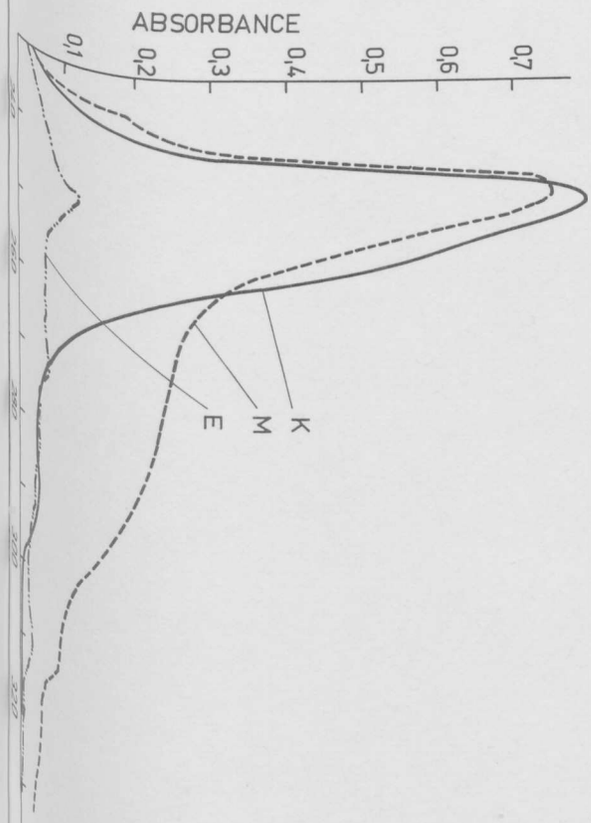

Fig. 3.

UV spectra of the colored substance fractions

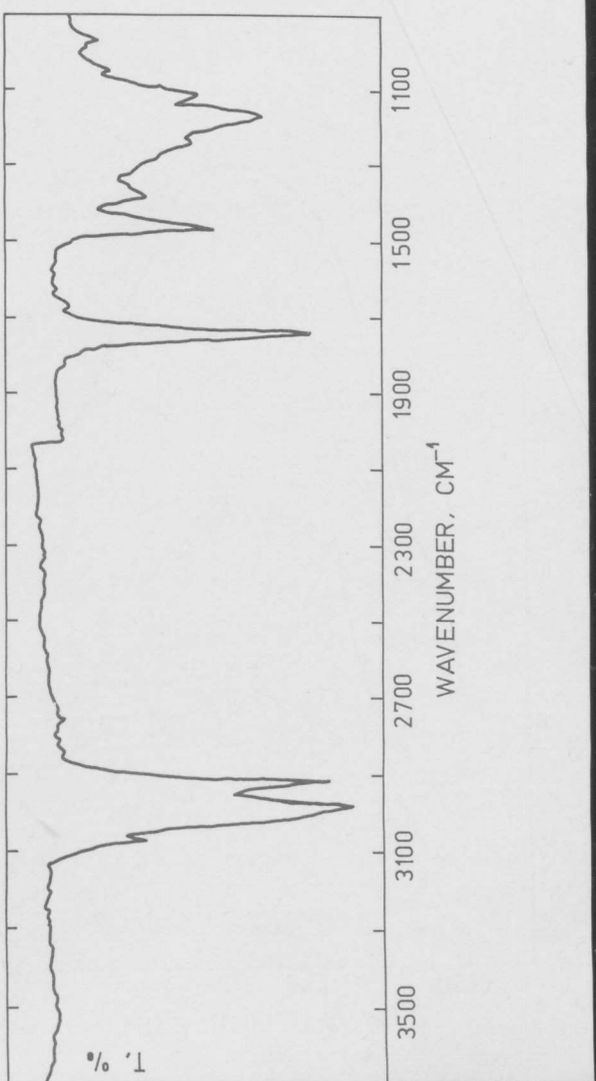

Fig. 4.

IR spectrum of fraction K

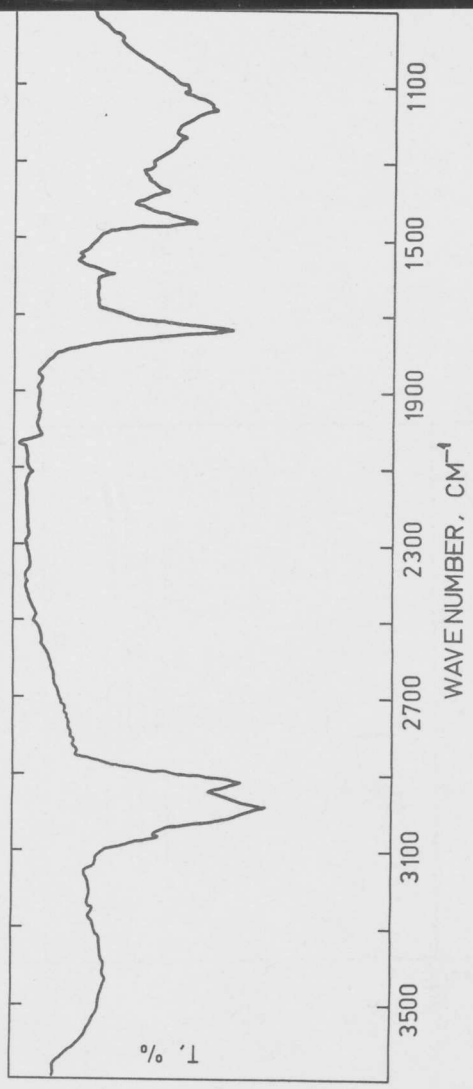

Fig.5.
IR spectrum of fraction M

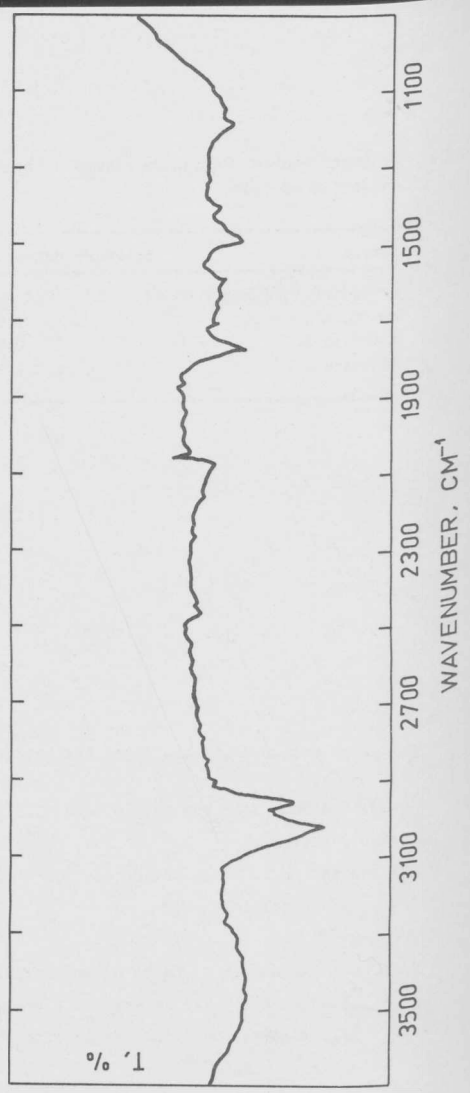

Fig. 6.
IR spectrum of fraction E

Fig. 7.
Fluorescent spectra of the colored subtance fractions