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EFFECT OF AUTOXIDATION OF RAPESEED OIL ON DEVELOPMENT OF MELANOPHOSPHATIDES

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The results obtained in the present study suggest that carbonyl compound developing in the course of autoxidation of rapeseed oil react with phosphatides, which, in turn, lead to development of coloured compounds, the so-called melanophosphatides, that reduce the quality of the phospholipids obtained from the oil.

The colour of commercial lecithin mainly depends on presence of brown-coloured compound [6, 7], which can develop during production or storage of the product. Natural colouring compounds affect the colour only marginally.

According to the literature [7], the brown coloured compounds, known as melanophosphatides, develop mainly as a result of reactions between aldehyde groups of sugars and amino groups of phospholipids.

It has been established that heating of phospholipids in oil in the presence of reducing saccharides causes development of browning compounds already at 60°C and this process is rapidly accelarated at higher temperatures. Ih the reaction some, bonded sugars may take part, for example, saccharo-aminic components of N-glucosides of certain phospholipids. Such compounds, just like melanoidins of aminoacids, isomerize at higher temperatures and transform into Shiff bases.

These, in turn, decompose into furfurol and oxymethylofurfurol. These compounds may react with aminoalcohols of phospholipides, thus giving melanophosphatides.

On processing of oil plant seed high temperature not only enhances the above reaction but also accelerates oxidation of unsaturated fatty acids, which ultimately leads to formation of aldehydes, for example.

In this paper there are described some attempts to specify the contribution of autoxidation reaction to formation of melanophosphatides, which reduce the quality of phospholipids.

It should be stressed that the presence of such compounds worsens not only the colour but also other organoleptic properties of phospholipide concentrates, that is, the taste and smell.

EXPERIMENTAL PART

The investigations were performed on model system. Solutions of rapeseed phospholipids were heated in oil subjected to oxidation to various content of aldehydes. Products of the reaction were analyzed by use of spectrophotometric method.

THE DETERMINATION OF PHOSPHOLIPIDS

In order to obtain possible unchanged rapeseed oil phospholipids a miscella from a fat processing plant was used as the primary raw material. Technological parameters of miscella distillation favour development of melanophosphatides, therefore the solvent was evaporated by destillation in a laboratory vacuum apparatus (pressure: 30 mm Hg; temp.: 45--60°C).

The oil which was yielded had Lea number 4.3 and acid number 3.6. The phospholipids were obtained by precipitation of compound from the oil at 4°C in nitrogen atmosphere [7]. After centrifuging, the phospholipids were purified in a silica-gel column [9]. Phospholipids were thus separated from oil and from such compounds as steroles, their esters, free fatty acids, natural coloured substances, and others.

Analysis of the eluates was carried aut by used thin-layer chromatography [5]. The phospholipids were then analyzed for the following properties: content of peroxides [4], phosphorus [1], acid number [4], sugar, before and after inversion [2]. From the phospholipids, which were used in further investigations, the sugers were removed (ether solution of phospholipids was treated with 50% ethanol solution [8]). The process was completed when no sugars were present in the water phase.

OXIDATION OF REFINED RAPESEED OIL

Rapeseed oil was oxidated to obtain samples of oil with various content of aldehydes. The apparatus used here is described elsewhere [2]. The process was conducted at 90°C, and at oxygen flow-rate 8-10 l/hr.

Lea number and aldehyde levels were determined in samples collected every 30 minutes. Aldehydes were determined by the used modified Henick method [3]. A calibration curve A = f (c) was plotted for capryl aldehyde with the use of VSU-1 spectrophotometer at 430 nm.

Samples of pure, no-sugar-containing phospholipids, as well as samples of oxidated oil of various aldehyde content were prepared.

REACTION OF PHOSPHOLIPIDES WITH MYRISTIN ALDEHYDE

A model reaction of myristin aldehyde with phospholipids was carried out. The following mixture was heated for 1 hour at 120°C in nitrogen atmosphere: 20 g paraffin oil, 0,4 g myristyl aldehyde and 0,3 g phospholipids. Spectrophotometric curves were plotted separately for myristyl aldehyde in paraffin oil, phospholipids in paraffin oil, and the mixture of phospholipids and myristyl aldehyde in paraffin oil. Paraffin oil was used as a reference.

REACTION OF PHOSPHOLIPIDES WITH OXIDISED OIL

10 g rapeseed oil with 5.6 mmole/kg aldehyde content was heated with 0,15 g phospholipids at 80°C for 1 hour. Nitrogen was flown through the mixture in order to prevent further oxidation changes in the oil. The quantity of phospholipids in a sample (1,5%) roughly corresponded to their content in the raw oil. Experiment was repeated with a sample of the same composition at 120°C, since the development of coloured products was very slow at 80°C, as it was indicated by spectrophotometric analysis. After every 30 min of heating a sample was collected and its absorption was measured in 0,01% chloroform solution within the range of 250-300 nm. Thus the dependence of absorption on heating time was determined. Then, 1,5% solutions of phospholipids in chosen samples of oxidated oil of different aldehyde content were prepared and heated at 120°C for two hours. Spectrophotometric curves were plotted for 0,01% chloroform sample solutions after the heating was completed.

During the spectrophotometric measurement the reference was always made to 0,01% chloroform oil solution oxidated to the same level as a given sample. In this way the absorption was eliminated which developed as a result of the presence of carbonyl compounds, which absorb roughly in the same range as melanophosphatides.

RESULTS AND DISCUSSION

The aim of the present study was to determine the proportion of compounds which develop during oil autoxidation and which influence the impairment of organoleptic properties of rapeseed phospholipids.

According to literature information, the browning of phospholipids is induced primarily by products of reactions of free sugars present. The investigations were therefore conducted with phospholipids devoid of such sugars. It was stated that presence of sugars was particularly undesirable since they react with phospholipides and produce melanophosphatides. The latter, mask the reaction of phospholipides with aldehydes derived from oxidated oil. The characteristics of phospholipids and the refined rapeseed oil used in the study is given in Table 1.

Table 1. Characteristics of refined rapeseed oil and phospholipides

Determination	Refined oil	Phospholipides
Acid number (LK)	0.38	16.8
Lea number (mMole O_2/kg oil)	1.4	0.92
Aldehydes (mMole/kg oil)	2.8	—
P %	—	2.45
Sugar before inversion %	—	0.43
Sugar after inversion %	—	6.7

The oil was subjected to accelerated autoxidation and samples at different levels of oxidation were obtained, that means the samples of different aldehyde contents. For further work only those samples were used which had Lea number roughly equivalent to that, present under technological conditions, expecially during oil storage. The samples ranged from Lea. 2.5 to Lea 30; there were two additional samples with higher Lea number. The contents of aldehydes (mmole/kg oil) is given in Table 2 (Samples 1-7). Another model reaction involved the heating of phospholipids and myristin aldehyde in paraffin oil matrix. It was aimed to identify the products of reaction spectrophotometricaly within 260-300 nm.

As can be seen in Fig. 1, Curves 1,2 and 3 have similar runs. They represent absorption of phospholipids, myristin aldehyde, and phospholipids after heating at 120°C respectively. The higher absorption of phospholipids heated at 120°C (Curve 3), as compared with the curve for non-heated phospholipids (curve 1), may be a result of the reaction of phospholipids with bonded sugars, which are not being eluated with 50% alcohol. Since the difference in absorption value of the two curves (1 and 3) at 278 nm was insignificant, it was not taken into account during interpretation of the results, particularly that the amounts of phospholipids in the tested samples were constant. None of the discussed curves shows a maximum between 260 and 350 nm. On the other hand, products of reaction of phospholipids with myristin aldehyde (curve 4) show a maximum at 278 nm, which corresponds according to literature [7] to absorpion of melanophosphatides.

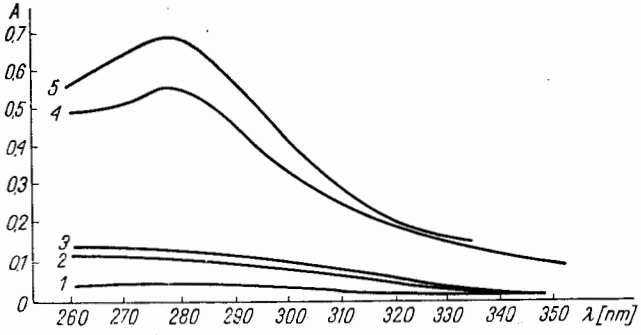

Fig. 1. Spectrophotometric curves of; 1 — phospholipides in paraffine oil, 2 — myristin aldehyde in paraffine oil, 3 — phospholipides in paraffine oil after heating at 120°C, 4 — products of reaction of phospholipides with myristin aldehyde in paraffine oil

The next step of research was aimed to show of whether the heating of phospholipids with oxidated oil produces melanophosphatides. Initial experiments showed that the absorption spectrum of these products provides a maximum absorption at 278 nm (Fig. 1, curve 5) that is, at a wavelength at which the maximum appears for products of condensation of myristyl aldehyde with phospholipides. Hence, a conclusion is derived that the final products of oil autoxidation, the aldehydes, react with amino groups of phospholipids and produce melanophosphatides. This was confirmed by subsequent experiments, which it was established that with the growing contents of aldehydes in a sample (Table 2), at a constant quantity of phospholipide level which corresponded to their avera-

Table 2. Content of aldehydes in oxidated rapeseed oil

Sample No.	Oxidation time; hr	Lea number; mMole O_2/kg oil	Content of aldehydes mMole/kg oil
1	0.3	2.5	3.9
2	2.0	3.4	5.6
3	8.0	15.4	8.6
4	13.0	25.4	10.9
5	16.0	30.0	14.0
6	19.5	55.0	28.1
7	20.5	95.0	78.8

ge contents in raw oil, the absorption of the reaction products increases (Fig. 2, curve 1-7). It should be stressed that absorption measurements have been carried out each time for 0,01% chloroform ample salution, hence, from the absorption increase it may be concluded of reaction pro-

duct concentration increase. It was determined also that the quantity of developing melanophosphatides depends largely on the heating time of the reaction mixture. This fact is illustrated by curve A = f(t) in Fig. 3,

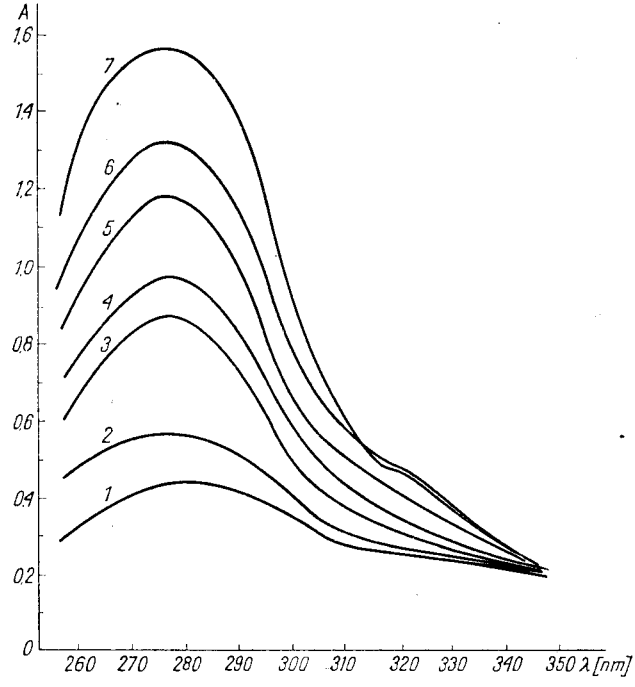

Fig. 2. Spectrophotometric curves of 0.1% chloroform solutions of products of reaction between phospholipides and oil samples with increasing content of aldehydes

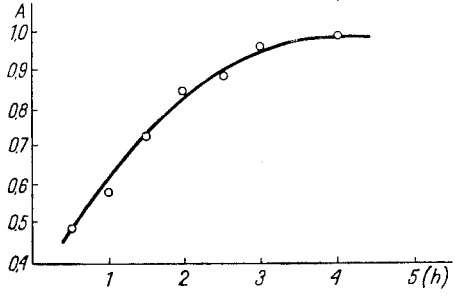

Fig. 3. Dependence of absorption at 278 nm 0.1% chloroform solutions of products of reaction between phospholipides and oxidated oil on heating time

which was plotted for sample 2 (Table 2). It can be observed, then, that absorption value grows within the first three hours of heating and then does not change practically.

The experiments proved that impairment of organoleptic properties of phospholipids, mainly change of colour, which is taking place during technological processing is caused by development of melanophosphatides. These compounds, as the present study indicated, develop not only in effect of a reaction between phospholipides and sugars, but also in condensation reaction of amino groups of phospholipides with aldehydes which form in the course of oil oxidation.

The more technological process condition enhances oil oxidation, the more brown-coloured compounds develop in commercial lecithin.

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WPŁYW AUTOOKSYDACJI OLEJU RZEPAKOWEGO NA POWSTAWANIE MELANOFOSFATYDÓW

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Streszczenie

Zbadano proces powstawania melanofosfatydów w wyniku kondensacji grup aminowych fosfolipidów z aldehydami pochodzącymi z utlenionego oleju. W tym celu otrzymano fosfolipidy i oczyszczono je z substancji towarzyszących, głównie wolnych

cukrów. Rafinowany olej rzepakowy poddawano procesowi utlenienia, a następnie próbki o różnej zawartości aldehydów ogrzewano w temperaturze 80 i 120°C z fosfolipidami. Wstępnie przeprowadzono reakcję fosfolipidów z aldehydem mirystynowym. Produkty reakcji badano spektrofotometrycznie w zakresie UV.

Ustalono, że proces autooksydacji, który towarzyszy wszystkim etapom wydobycia i rafinacji oleju ma swój udział w powstawaniu melanofosfatydów. Podobnie jak podczas reakcji fosfolipidów z cukrami, w obecności aldehydów pochodzących z utlenionego oleju, powstają barwne związki pogarszające właściwości organoleptyczne fosfolipidów.