Vol. XII (XXXVI), No. 3—4 1986

INA BRATKOWSKA
WŁODZIMIERZ ZWIERZYKOWSKI

COMPARISON OF COMPLEX FORMING POWER OF RAPESEED PHOSPHOLIPIDS AND OF CITRIC ACID DURING OIL AUTOXIDATION

Institute of Organic and Food Chemistry and Technology,
Technical University, Gdańsk

Key words: rapessed oil, autoxidation, antioxidants, phospholipids.

Complex forming power of phospholipids with copper ions appearing in refined rapeseed oil as related to the formation of citric acid copper complexes were investigated. The curves of the autoxidation process were plotted and the influence of forming power of both components was compared. It was found that phospholipids form compexes with metallic ions similarly as citric acid and that the mechanism of complex formation of phospholipids plays a substantial role in the antioxidative activity of these compounds.

INTRODUCTION

One of the causes of fat oxidation are heavy metals remaining in the fats after their technological processing. Copper ions are catalytically active at room temperature when their content is 0.1-1 μg/g [9]. The autoxidation due to the presence of heavy metals is inhibited by substances inactivating catalitic action of these metals by forming complexes or chelating with them. Among the known and practically used substances of this kind are acids such as the citric, the ascorbic, the folic and the phytic acids. According to some authors [7], phospholipids too are capable of forming complexes with metals. Vegetable phospholipids which are complex mixtures of compounds often dosplaying different chemical properties due to different functional groups in molecules of the various compounds, are seen as having both antioxidative and synergetic properties [1, 2, 4, 8, 15, 16]. The mechanism of phospholipid activity is not fully clarified. Phospholipids are not included among typical antioxidants acting according to the radical mechanism [19, 24]. It is even suggested that the decisive factor are products of phospholipid transformation, namely Maillard's brown compounds [11, 12, 21]. The synergetic activity of phospholipids in the presence of natural antioxidants such as tocopherols was demonstrated by Hildebrandt et al. [5]. According to these authors, the strong synergetic effect of purified phospholipids, having considerable surface energy, suggests the action of surface processes on the lipids-air phase boundary. The synergetic properties of phospholipids in the presence of metals were also investigated by El-Tarras et al [3] who found that fresh phospholipids inhibit the autoxidation of methyl esters of cottonseed oil and reduce the prooxidative action of metals. Rutkowski [23] claims that the protective effect of rapeseed oil phospholipids is due to their bonding of iron. Hence, the lower the phospholipid content in oil, the more drastic the prooxidative effect of this metal. In this work we compared the complex forming power of rapessed oil phospholipids and citric acid as regards the heavy metals present in the oil in order to elucidate the role of these reactions in the complex mechanism of autoxidation inhibition by phospholipids.

MATERIAL AND METHODS

Rapeseed phospholipids, refined rapeseed oil, copper stearate and citric acid were used to compare the complex forming power of rapeseed phospholipids and citric acid.

1. The phospholipids were obtained from post-hydration gums containing 40% water, 45% phospholipids and 15% oil from the Gdańsk Fat Industry Enterprise. The processing of the gums involved two operations:

— drying in a vacuum drier at $19.6 \cdot 10^3$ Pa and 60°C, and

— deoiling of the dried residues with acetone cooled down to 5°C [20].

The obtained phospholipids were in the form of yellow powder; they were stored at −15°C in nitrogen atmosphere. The characteristic of the phospholipids is given in Table 1. The composition of fatty acids of the phospholipids was determined by gas chromatography. The phospholipids were subjected to estrification [10] and the methyl esters were chromatographed on a 3-m column filled PEGA+DGS (2:1) placed on a chromosorb W 80-100 mesh, at 195°C. The results are shown in Table 2. The phospholipids composition was determined by thin layer chromatography using the eluant chloroform-methanol-water (65:24:4 by vol.) [17]. The various compounds were identified using colour reactions [22] and R coefficients given in the literature [17]. Preparative thin layer chromatography was applied to determine the percent content of the different compounds in the phospholipids mixture. The phospholipids were dissolved in chloroform in a 1:1 proportion and applied in the form of bands onto plates covered with silica gel with a semi-automatic applicator. After

Table 1. Characteristic of rapeseed oil phospholipids

Chemical test	Average value
Acid value (in benzene)	30.5
Acid value (in acetone)	0.2
Peroxide value	3.0

Table 2. Fatty acid composition of rapeseed oil phospholipids and refined rapeseed oil

Fatty acid	I %	II %
$C_{16:0}$	11.2	2.7
$C_{16:1}$	1.0	0.4
$C_{18:0}$	1.9	0.6
$C_{18:1}$	21.7	12.8
$C_{18:3}$	6.2	8.8
$C_{20:1}$	6.9	8.6
$C_{22:1}$	27.5	53.4

Table 3. Composition of rapeseed oil phospholipids

Component	%
Polyphospholipids Phosphatidic acids	2.0
Phosphatidylethanolamine	17.9
Phosphatidylopholine	29.5
Inositol phosphatide Phosphatidylserine	28.6
Lysophosphatide	17.6
Other compounds without phosphorous	0.3

developing the chromatogram, the content of the separate compounds in the mixture was determined by the grawimetric method. The composition of rapeseed phospholipids is given in Table 3.

2. The refined rapeseed oil with acid value 0.3 and peroxide value 3.0 was obtained from the Gdańsk Fat Industry Enterprise. The fatty acid composition of this oil is given in Table 2.

3. The copper stearate was obtained by bringing to boiling a water solution of $CuSO_4 \cdot 5H_2O$ with stearic acid. Stoichiometric quantities of substrates were taken for the reaction. A product insoluble in water was obtained, and it was washed several times with hot water in order to remove the $CuSO_4$ that did not enter the reaction, and the H_2SO_4 formed

during the reaction. The copper stearate was dried in a desiccator with a drying agent, and the copper content in it was calculated as the difference between copper taken for reaction and copper determined iodometrically [13] in the residue. This content was $4.77 \cdot 10^{-2}$ g Cu/g copper stearate.

4. Chemically pure commercial citric acid was used.

Autoxidation of rapeseed oil with additions of copper and citric acid, and copper and rapeseed phospholipids was performed. The experiment was carried out in 300 cm^3 vessels equipped with a sintered glass plate and pipe feeding oxygen in the bottom part. The vessels were placed in a thermostat; reaction temperature was 70°C, and oxygen flow was 9-10 l/h. Sample weight was 150 g. During oxidation samples were taken for determination of the peroxide value. The obtained results are shown as autoxidation curves coupled in pairs: one of the curves represents the oxidation of 0.0005% Cu^{2+} in rapeseed oil in the presence of citric acid, and the other — in the presence of phospholipids; the number of phospholipid moles per one Cu^{2+} ion is always twice that of the number of citric acid moles per one Cu^{2+} ion. The concentrations of phospholipids and citric acid are expressed as the number of moles of these compounds per one Cu ion.

DISCUSSION OF RESULTS

The experiments were performed with highly purified rapeseed phospholipids obtained from posthydration gums in order to avoid the negative effect on the quality of these compounds of the drastic drying conditions in industrial processing. The composition and characteristic of the phospholipids are given in Tables 1 and 3. Compared to refined rapeseed oil, the phospholipids contain a relatively large amount of unsaturated fatty acids (Table 2).

The phospholipids' complex forming power with relation to metals that might be present in oils (using Cu ions as an example) were studied by comparing the effectiveness of autoxidation inhibition by the phospholipids and by citric acid, a typical complex forming compound. In the first series of experiments citric acid was added to a copper stearate solution in refined rapeseed oil (0.0005% Cu concentration), while in the second series the addition was that of rapeseed phospholipids. The solutions were oxidized in the presence of oxygen at 70°C, and the progress of oxidation was expressed as the peroxide value. The concentrations of Cu^{2+} in the oil with citric acid addition were 1, 3, 5, 10, 15 and 20 moles citric acid per one Cu ion. The autoxidation curves plotted on the basis of the obtained results are presented in Figs 1-6 (curves 1, 3, 5, 7, 9 and 11), and Table 4 gives the time required for the studied solutions to attain a peroxide value

Table 4. The time necesarry for the Cu^{2+} solution in refined rapeseed oil to reach a peroxide value

Mole citric acid/Cu^{2+}	Time h	Mole phospholipids/ /Cu^{2+}	Time h
1	2.5	2	2.5
3	3.0	6	3.0
5	4.0	10	4.5
10	5.5	20	6.0
15	6.0	30	7.0
20	7.0	40	8.0

of about 12. As can be seen from this Table, this time increases with the increase of citric acid concentration, which means that the inhibition of autoxidation is greater when the concentration of the complex forming compound increases. The course of the autoxidation curves, and in particular the length of the induction period, confirms this claim (Figs 1-6).

In the second series of experiments, the concentrations of the Cu^{2+} solutions containing phospholipids were 2, 6, 10, 20, 30 and 40 moles of phospholipids per one Cu ion. The following assumptions were made to facilitate the interpretation of results:

1) the molar concentration of phospholipids was converted to mean molecular weight of phosphatidic acid,

2) four phospholipids molecules are bonded by one Cu^{2+} ion. The first assumption is made with a reservation, namely the exact determination of the molarity of phospholipids is impossible since they are combinations of compounds with various molecular weight. Hence, the calculated molar concentrations of the phospholipids were certainly higher than the real concentrations of these compounds. As for the second assumption, it was presumed that it is the phosphate groups that are active in the formation of complexes with Cu, and that the Cu ion, having coordination number 4, will bond four phospholipid molecules. One must add that such an active group may also be the carboxyl group presents in serine. However, it may be assumed with a measure of probability that copper forms complexes in which there are four phospholipid molecules for every Cu^{2+} ion. The autoxidation curves of phospholipid solutions in refined rapeseed oil with Cu^{2+} ion additions are shown in Figs 1-6 (curves 2, 4, 6, 8, 10 and 12). In this case too the increase of phospholipids concentration extends the induction period. All six of the obtained curves have induction periods lying between the induction period for refined rapeseed oil (curve 13 on Fig. 6) and for oil with a copper addition (curve 0 on Fig. 1). Table 4 gives the time of autoxidation necessary for the studied oil solution with Cu^{2+} ions and phospholipids to attain a peroxide value of about 12. This time distinctly increases with the increase of phospholipids concentration. To

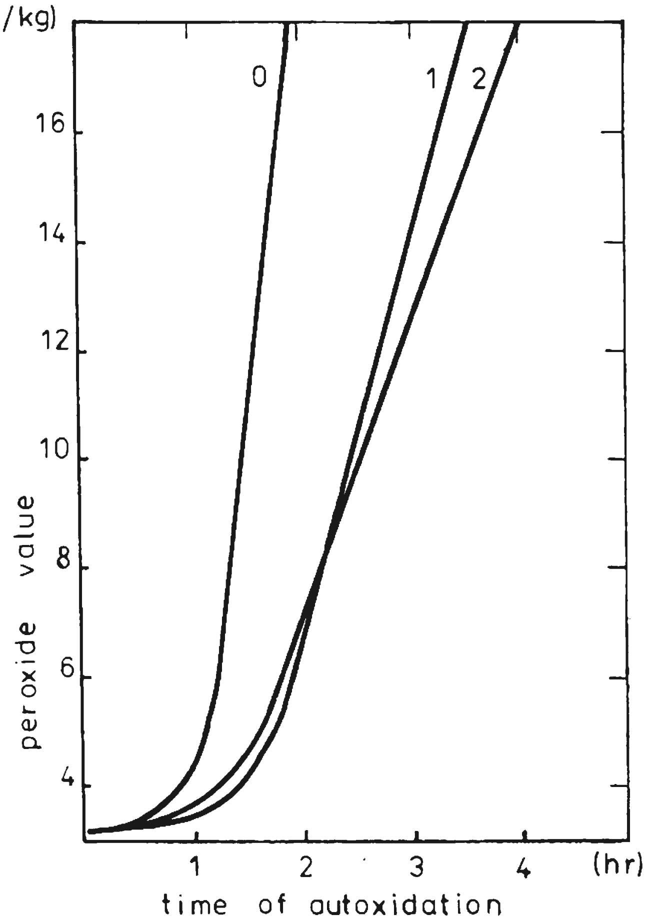

Fig. 1. Autoxidation of 0.0005% Cu^{2+} solution in refined rapeseed oil with addition of citric acid and phospholipids. Curve 0—0.0005% Cu^{2+} solution in refined rapeseed oil; 1 — 1 mole citric acid/Cu^{2+}, 2 — 2 moles phospholipids/Cu^{2+}

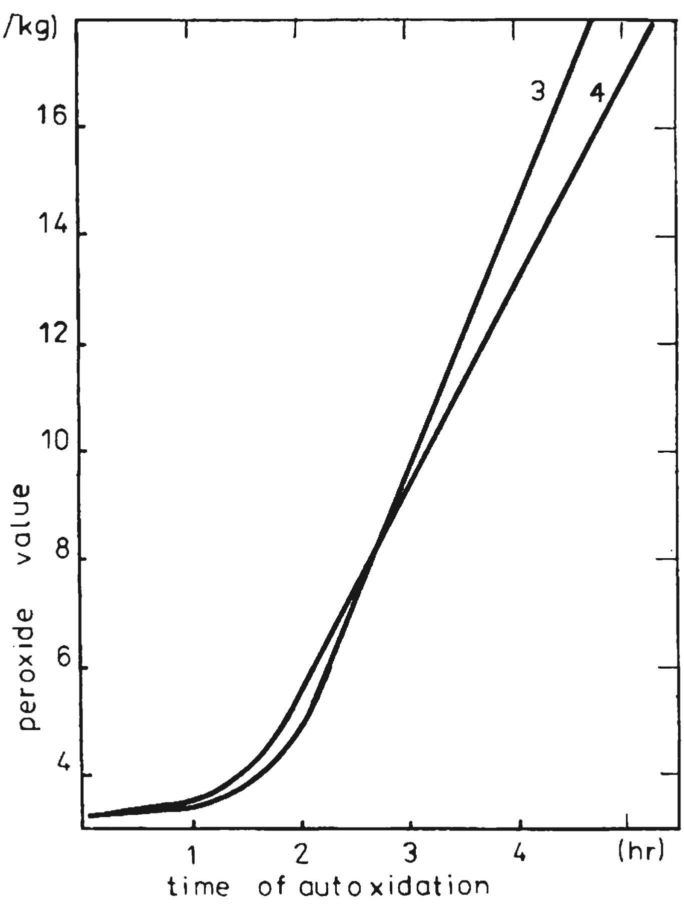

Fig. 2. Autoxidation of 0.0005% Cu^{2+} solution in refined rapeseed oil with addition of citric acid and phospholipids. Curve 3 — 3 moles citric acid/Cu^{2+}, 4 — 6 moles phospholipids/Cu^{2+}

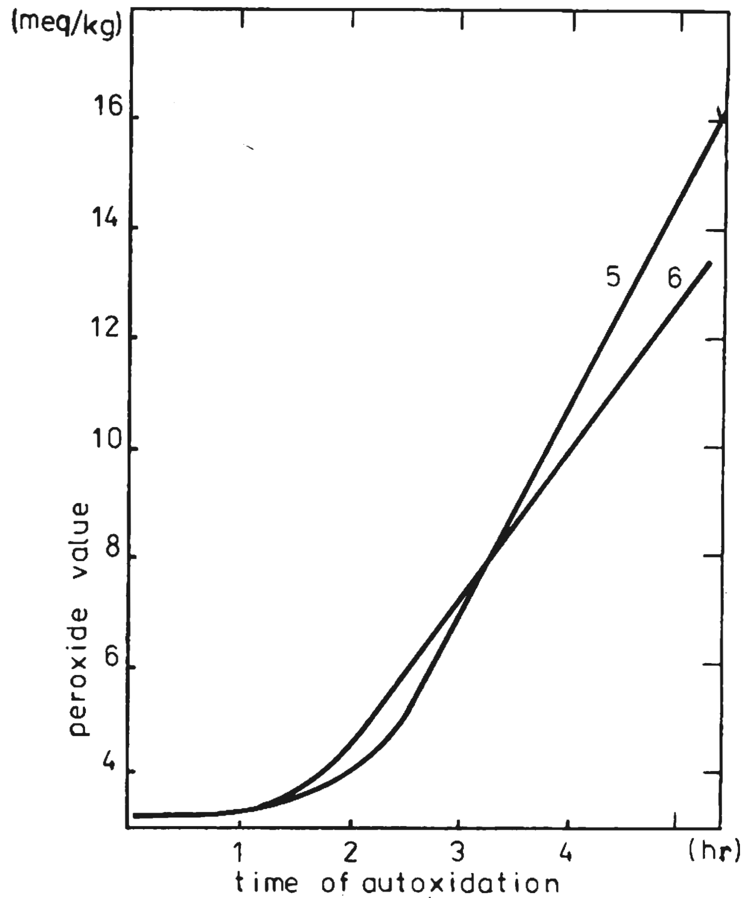

Fig. 3. Autoxidation of 0.0005% Cu^{2+} solution in refined rapeseed oil with addition of citric acid and phospholipids. Curve 5 — 5 moles citric acid/Cu^{2+}, 6 — 10 moles phospholipids/Cu^{2+}

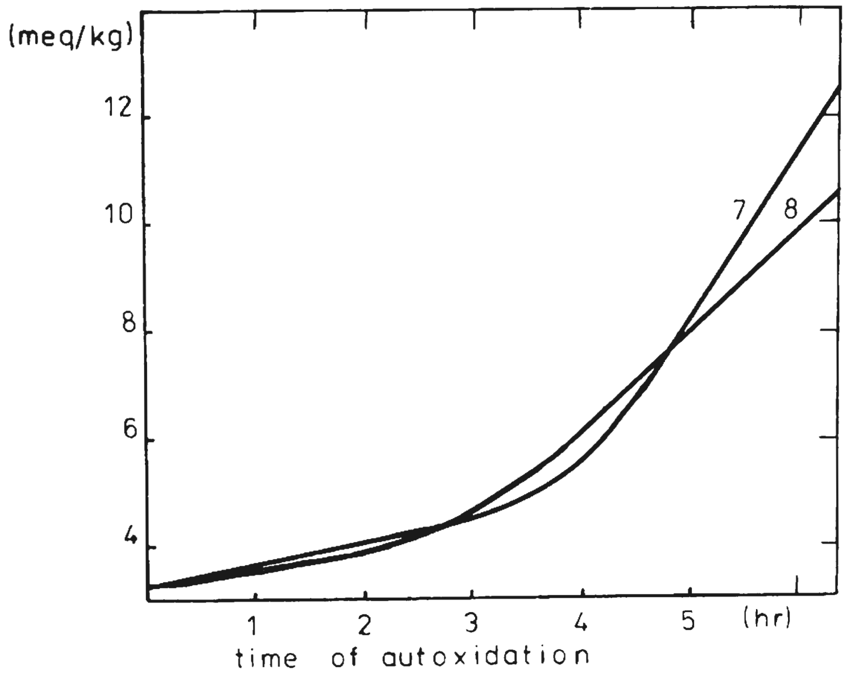

Fig. 4. Autoxidation of 0.0005% Cu^{2+} solution in refined rapeseed oil with addition of citric acid and phospholipids. Curve 7 — 10 moles citric acid/Cu^{2+}, 8 — 20 moles phospholipids/Cu^{2+}

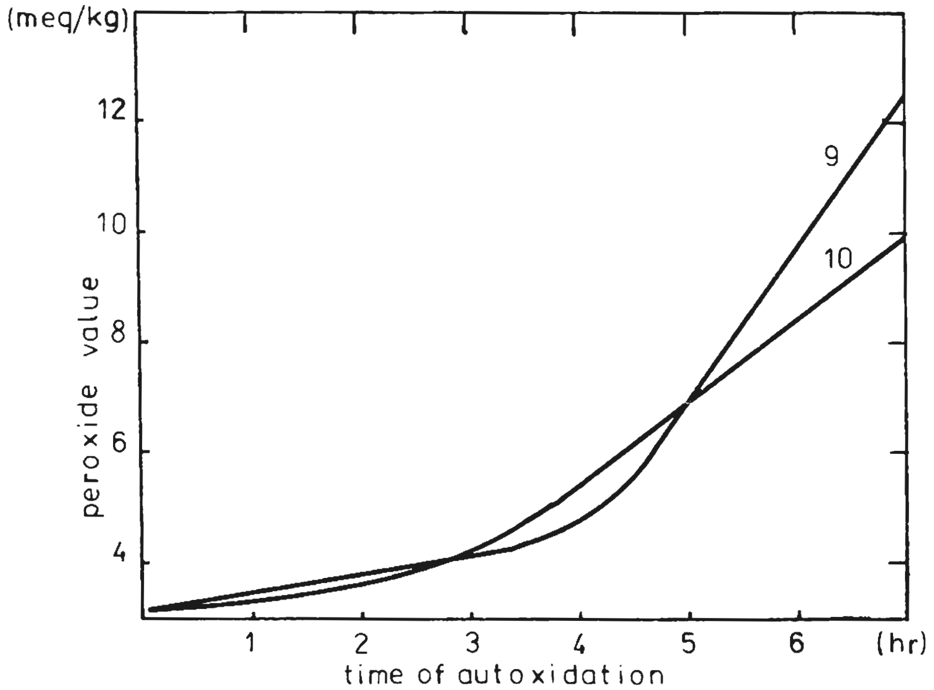

Fig. 5. Autoxidation of 0.0005% Cu^{2+} solution in refined rapeseed oil with addition of citric acid and phospholipids. Curve 9 — 15 moles citric acid/Cu^{2+}, 10 — 20 moles phospholipids/Cu^{2+}

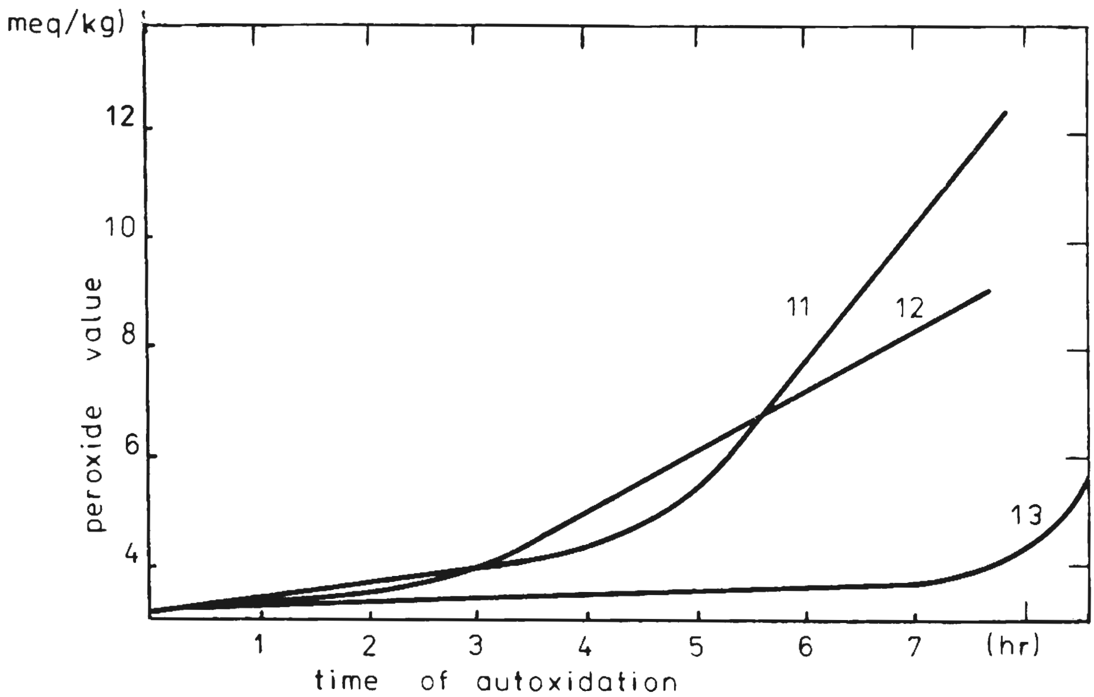

Fig. 6. Autoxidation of 0.0005% Cu^{2+} solution in refined rapeseed oil with addition of citric acid and phospholipids. Curve 11—20 moles citric acid/Cu^{2+}, 12 — 40 moles phospholipids/Cu^{2+}, 13 — refined rapeseed oil

enable a comparison of the complex formation properties of citric acid and the phospholipids, the respective curves are shown in pairs with each pair representing such concentrations of these compounds which ought to give similar complex formation effects (Figs 1-6).

It was assumed here that a certain number of citric acid molecules ought to bond as many Cu ions as the twice higher number of phospholipids molecules since, as assumed previously, for every Cu ion in the complex there are two molecules of citric acid or four molecules of phospholipids. This assumption was confirmed in practice. The course of all the pairs of autoxidation curves is similar. However, not all sections of the curves overlap. It can be seen from curves 1-6 (Figs 1-3) that at lower concentrations of both citric acid and phospholipids (up to peroxide values of about 8) the complex formation effect of citric acid is slightly greater, while at peroxide values in excess of 8, the phospholipids become more effective. At higher concentrations of both the complex forming components (Figs 4-6) the autoxidation curves coincide up to peroxide value 4; in the 4-7 range of peroxide values the effect of citric acid is greater, and at peroxide values over 7 the phospholipids become the more effective antioxidation agents. The better antioxidation effect of phospholipids at hogher peroxide values may be explained by the fact that another antioxidation machanism of the phospholipids comes into play when there is a large accumulation of autoxidation products, e.g. the antioxidation action of the Maillard's reaction products which may have been formed as a result of the reaction between aldehydes, secondary autoxidation products, and phospholipids which contain first-order amine groups. And this is responsible for the more effective action of phospholipids.

CONCLUSIONS

1. The complex forming power of phospholipids in relation to heavy metal ions are similar to those of citric acid.
2. The metal-bonding mechanism in the action of phospholipids plays a significant role in the antioxidation effect of these compounds.

LITERATURE

1. Bishov S. J., Henick A. S., Koch R. B.: Food Research, 1960, **25,** 174.
2. Bratkowska I., Niewiadomski H.: Acta Alim. Polonica 1975, **25,** 339.
3. El-Tarras M., Fayfz M., Moety Ezzat M. Abbel, Ahmad Abdel Kader S., Amer M. M.: Oleagineus 1979, **34,** 139.
4. Evans C. D., Conney, P. M., Scholfield C. R., Putton H. J.: Am. Oil Chemists Soc., 1954, **31,** 295.
5. Hildebrandt D. H., Teraw J., Kito M.: J. Am. Oil Chemists Soc., 1984, **61,** 552.
6. Jakubowski A., Rokosz K.: Tłuszcze i Środki Piorące 1958, **4,** 232.
7. Korniena E. P., Litwinowa E. D., Arutunian N. S.: Masło-Żirowaja Promyšlennost 1978, 5, 12.
8. Luchmann F. H., Melnick D., Miller J. D.: J. Am. Oil Chemists Soc., 1953, **30,** 602.
9. Marcinkiewicz S.: Tłuszcze, Środki Piorące 1972, **16,** 3.
10. Matcalfe L. D., Schitz A. A., Pelke J. R.: Anal. Chem., 1966, **38,** 514.
11. Masaymki Maruyama, Kenshiro Fujimoto, Takashi Kaneda, Nippon Shokumin, Kogyo Gakkaishi, 1970, **17,** 281.

12. Mieth C.: Dissertation, Math-Nat. Fakultet, Humbolt Universität zu Berlin 1968.
13. Minczewski J., Marczenko Z.: Chemia Analityczna. Analiza Ilościowa, PWN, Warszawa 1978, 292.
14. NB-81 8054-01.
15. Olcott H. S., der Veen J. V.: J. Food Sci., 1963, **28**, 313.
16. Paquot C., Mercier M. J.: Rev. France Corps Gras, 1963, **10**, 337.
17. Persmark U.: J. Am. Oil Chemists Soc., 1968, **45**, 742.
18. PN-60/A-86918.
19. Pokorny J., Poskocilova H., Davidek J.: Die Nahrung 1981, **25**, (6), K29-k31.
20. Rewald B.: Patent W. Brytania, 412224, 1932.
21. Rżechin W. P., Preobrażenskaja I. S.: Masło-Żirowaja Promyšlennost 1959 (7), 20.
22. Pod red. Rżechina W. P., Siergiejewa A. G.: Rukowodstwo po metodam issledowanija, technochimičeskomu kontrolu i učetu proizwodstwa w masłożirowoj promyšlennosti. WNIIŻ, Leningrad 1967, t. I, 316.
23. Rutkowski A.: Fette, Seifen, Anstrichmittel 1959, **61**, 1216.
24. Taufel K., Kretzschmann F., Francke Cl.: Fette, Seifen, Anstrichmittel 1960, **62**, 1061.

Manuscript received: April, 1985
Authors address: 80-952 Gdańsk, Majakowskiego 11

I. Bratkowska, W. Zwierzykowski

PORÓWNANIE WŁAŚCIWOŚCI KOMPLEKSOTWÓRCZYCH FOSFOLIPIDÓW RZEPAKOWYCH I KWASU CYTRYNOWEGO W PROCESIE AUTOOKSYDACJI OLEJU

Instytut Chemii i Technologii Organicznej oraz Żywnościowej, Politechnika, Gdańsk

Streszczenie

Zbadano właściwości kompleksotwórcze fosfolipidów rzepakowych w stosunku do metali mogących występować w olejach roślinnych na przykładzie jonów miedziowych. W tym celu porównano przebieg procesu autooksydacji 0,0005% roztworu miedzi w rafinowanym oleju rzepakowym z dodatkiem fosfolipidów i oddzielnie z dodatkiem kwasu cytrynowego — typowego związku kompleksotwórczego. Przyjęto, że jeden jon Cu^{2+} wiąże dwie cząsteczki kwasu cytrynowego i cztery cząsteczki fosfolipidów. Przebieg krzywych autooksydacji potwierdził to założenie (rys. 1-6). Fosfolipidy wykazują podobny do kwasu cytrynowego efekt przy stężeniach molowych tych związków dwukrotnie wyższych niż kwasu cytrynowego. Np. krzywe autooksydacji roztworu miedzi w rafinowanym oleju rzepakowym z dodatkiem jednego mola kwasu cytrynowego (Cu^{2+} i dwóch moli fosfolipidów) Cu^{2+} (rys. 1, krzywe 1 i 2) lub pięciu moli kwasu cytrynowego (Cu^{2+} i dziesięciu moli fosfolipidów) Cu^{2+} (rys. 3, krzywe 5 i 6) mają podobny przebieg. Przy wyższych wartościach liczby nadtlenkowej (powyżej 7) przeciwutleniający efekt fosfolipidów jest większy, co można wytłumaczyć tym, że przy dużym nagromadzeniu produktów autooksydacji włącza się inny mechanizm przeciwutleniającego działania fosfolipidów.