EXAMINATION OF THE PROTECTIVE EFFECT OF TOCOPHEROLS ON PROVITAMIN A (ß-CAROTENE) AND VITAMIN A IN ENRICHED MARGARINE AND BUTTER

By

L. TELEGDY KOVÁTS and E. BERNDORFER-KRASZNER

Department of Food Chemistry, Polytechnical University, Budapest

Received October 3, 1966

Taking into account the chemical properties of tocopherols, 6-hydroxy-chromane derivates, the mode of vitamin E action is probably mostly based on the following two explanations:

1. as intracellular antioxydants they exert a protective effect by inhibiting the autoxydation of vitamin A in unsaturated fats and similar natural organic substances of vital importance, sensitive to oxygen, such as ascorbic acid; and (or)

2. as electron carriers, they take part in respiration, biological oxydation and other vital processes. Vitamin effect is thus essentially bi-directional and may have an antioxydant effect and an enzyme effect. In this respect the tocopherols, considered initially as inductive vitamins, are near to the prosthetic vitamins [1].

With regard to food chemistry and industry, the tocopherols have a particularly interesting and significant property: as natural anti-oxydants they inhibit the oxydative rancidity of fats and fatty foodstuffs.

A large number of researchers have examined in vivo the protective effect of tocopherols on provitamin A (β-carotene) and vitamin A [2], but only comparatively few analyses have been undertaken to control in vitro this economically important protective effect.

In our work we have studied in vitro the antioxydative and protective effect of tocopherols in margarine enriched with provitamin A (β-carotene) and in butter containing natural vitamin A. Enriched with provitamin A (β-carotene), margarine is a current and up-to-date foodstuff in several countries; its general use is justified by its fine colour similar to that of summer butter and by its biological value. BAUERFEIND et al. [3] have developed the technology of colouring fatty nutritive substances; they used the vegetable oil suspension of micro-pulverized β-carotene for colouring margarine. KOEHN [4] and BURNS et al. [5] have conducted biological analyses for controlling the degree of conversion in animals. They have found that the effect of β-carotene conversion in rats daily receiving 1 mg of tocopherol is as active as that of vitamine A.

The nutritive value of butter is increased by its richness in vitamin A. However, the vitamin A contents of butter shows a considerable fluctuation in the different seasons [6]. It would be an important task to keep the vitamin A content at a uniform level in spring and summer.

As antioxydants we have used α- and γ-tocopherol (Kochlight Lab., Ltd. Bolnbrook, Bucks.) as well as tocopherol concentrate extracted from maize germ oil. The preparation had 0,10% of tocopherol contents, including 80% of γ-tocopherol. For examining the behaviour of synergists, we have chosen citric acid, phosphoric acid and selenious acid. Several researchers [7, 8] have recently dealt with the protective effect of selenium compounds on tocopherols, but not with their utilization in food chemistry. We have therefore carried out experiments also to detect the mode of action of selenium in connection with the synergism of selenious acids on the anti-oxydant effect exerted by tocopherols. Our examinations were of informative character, their utilization in food chemistry is still subject to preliminary toxicological control. The aim of these examinations was to find out in the course of autoxydation

which tocopherol extends the induction period longer than any other,

which tocopherol has the most efficient protective effect on β-carotene and vitamin A,

and finally which synergist increases the anti-oxydative effect most efficiently.

We have studied accordingly the antioxydative properties of tocopherols

1. at autoxydation produced by ultra-violet irradiation,
2. on the application of the Active Oxygen Method [16].

Due to artificial ultra-violet irradiation the fats are a site of processes similar to those presumably taking place in oils, lard or margarine kept in unprotected shopwindows. The apparatus used for ultra-violet irradiation has an output of 220 $\pm$ 15 W. The samples were placed in Petri dishes of 8 cm diameter, in layers of 8 mms and kept at a distance of 16 cms from the ultra-violet lamp. The temperature during irradiation was of 25 $\pm$ 2 °C; time of irradiation: 1—3—5 hours. After irradiation, the samples were kept in dark for 24 hours, and the analyses were carried out subsequently. The Active Oxygen Method was applied according to the standards. We have measured the time required for achieving the peroxide value of 62,5 mmol /kg of the test substance under the special circumstances of the method. The length of this time can be taken as an index of resistance to rancidity. In every case we have determined the value of the autoxydation factor too. Our experiments included the determination of the peroxide number and of eventual contents in anti-oxydants, metal traces and vitamins (provitamin A, vitamin A and E).

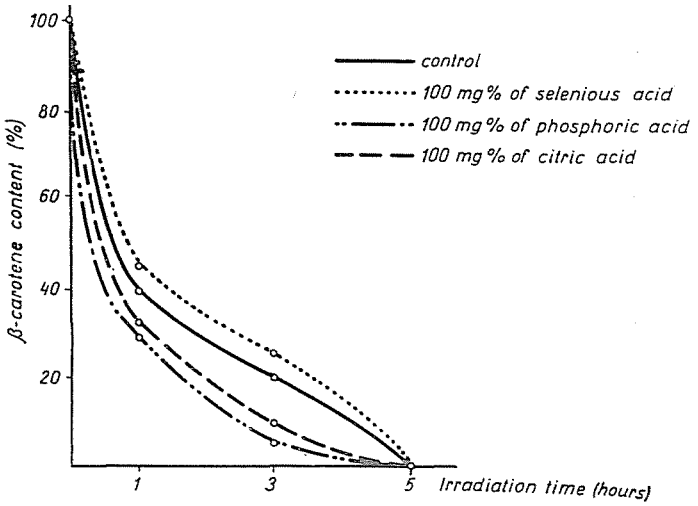

Fig. 1. Effect of synergents on the β-carotene content of enriched margarin during UV-irradiation

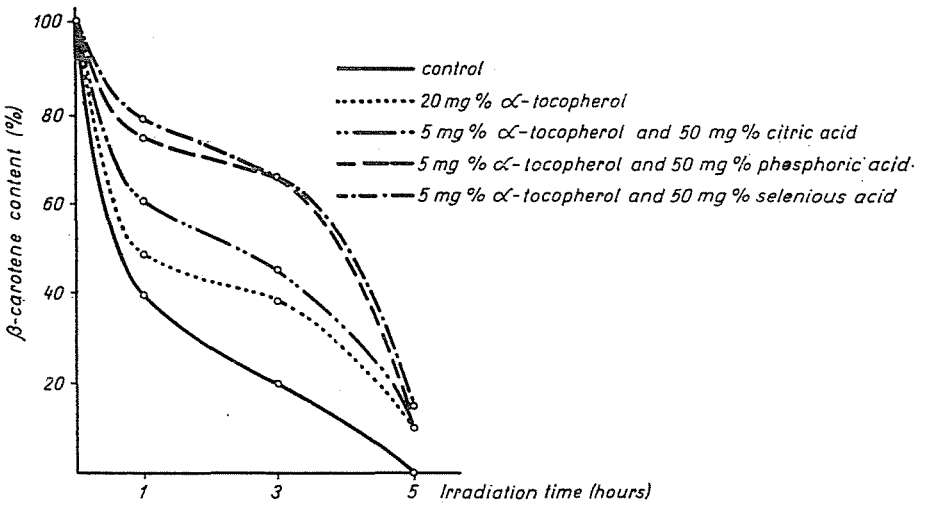

Fig. 2. Effect of α-tocopherol on the β-carotene content of enriched margarin during UV-irradiation

The peroxide number was determined according to LEA [9]. The detection of synthetic antioxydants was made with BIEFER's paper-chromatography [10]. This technique has the advantage of not extracting from the substrate the tocopherols present in their natural form. The iron(III) compounds were traced with the thiocyanate method of JACOBS [11], the copper (II) compounds with the dithizone method of MONIER-WILLIAMS [12] and the aluminium (III) compounds with the titrimetric method of JACOBS [11]. We have

Table 1

Effect of tocopherols and some acid synergents in

Tocopherols	Conc. mg/100 g	Acid synergents	Conc. mg/100 g	Peroxide value mmol/kg in margarin			
				Originally	Irradiation time		
					1	3	5
—	—	—	—	2.00	30.00	75.00	120.00
	50.00	—	—		14.00	38.00	70.00
α	20.00	—	—	2.00	10.00	30.00	55.00
	10.00				18.00	46.00	80.00
	50.00				20.00	47.00	76.00
γ	20.00	—	—	2.00	15.50	40.50	74.00
	10.00				26.00	50.00	84.00
	50.00				10.00	27.00	68.00
mixture	20.00	—	—	2.00	8.00	22.00	60.00
	10.00				14.00	33.00	76.00
		citric			11.00	32.00	40.00
α	5.00	phosphoric	50.00	2.00	10.00	28.00	35.00
		selenious			8.00	20.00	28.00
		citric			20.00	38.00	60.00
γ	5.00	phosphoric	50.00	2.00	14.00	32.00	52.00
		selenious			10.00	24.00	40.00
		citric			6.00	18.00	30.00
mixture	5.00	phosphoric	50.00	2.00	6.00	12.00	28.00
		selenious			5.00	10.00	20.00

worked out a rapid method to be used in fats for the determination of β-carotene, based on the direct extraction of provitamin A by solvents, its isolation from interfering substances by column chromatography and on photometry [13]. Based on the CARR-PRICE reaction, the method of MOOR [14] was used for the determination of vitamin A. The qualitative and quantitative determination of tocopherols in natural organic substances was made with a method of thin-layer chromatography we have developed [15]. The test material was margarine not enriched with vitamins, which we analyzed for possible peroxides, tocopherols, β-carotene and antioxydants and metal traces. Experimental butter was subjected to the same analyses. Margarine was coloured by crystalline β-carotene dissolved in sunflower oil (Light, Lab., Ltd. Colnbrook). β-carotene has at room temperature an approximative solubility of 0,08%

enriched margarin and butter during UV-irradiation

Peroxide value mmol/kg in butter				%-content of β-karotene				%-content of A-vitamin			
Originally	Irradiation time			Originally	After hours of irradiation			Originally	After hours of irradiation		
	1	3	5		1	3	5		1	3	5
0.40	1.80	17.00	31.50	100.00	40.00	20.00	—	100.00	40.00	12.00	—
	0.80	7.00	14.00		45.00	30.00	8.00		70.00	53.00	24.00
0.40	0.50	1.90	3.10	100.00	50.00	37.00	10.00	100.00	70.00	61.00	32.00
	1.00	10.00	22.00		38.00	25.00	5.00		60.00	45.00	10.00
	1.00	10.00	20.00		40.00	25.00	3.00		60.00	32.00	12.00
0.40	0.85	5.00	10.50	100.00	46.00	30.00	2.00	100.00	64.00	48.00	20.00
	1.10	18.00	28.60		32.00	22.00	—		52.00	25.00	5.00
	0.60	5.00	12.00		60.00	35.00	10.00		62.00	45.00	18.00
0.40	0.50	1.60	3.00	100.00	65.00	40.00	14.00	100.00	66.00	50.00	25.00
	1.00	8.00	19.00		40.00	30.00	8.00		55.00	32.00	8.00
	0.60	2.00	3.00		60.00	45.00	10.00		70.00	65.00	38.00
0.40	0.50	1.80	2.80	100.00	75.00	65.00	10.00	100.00	72.00	68.00	40.00
	0.40	1.50	2.40		80.00	65.00	15.00		80.00	70.00	44.00
	0.80	4.00	7.0		50.00	30.00	5.00		65.00	52.00	22.00
0.40	0.72	3.00	6.50	100.00	54.00	40.00	6.00	100.00	67.00	55.00	26.00
	0.60	2.50	4.00		60.00	54.00	10.00		70.00	60.00	30.00
	0 50	1.50	2.80		70.00	52.00	20.00		68.00	57.00	30.00
0.40	0.50	1.30	2.50	100.00	70.00	55.00	25.00	100.00	69.00	60.00	35.00
	0.40	1.10	1.90		75.00	60.00	30.00		75.00	66.00	40.00

in vegetable oils. The solution was kept in a brown polished glass-stoppered flask, in a refrigerator at ± 2 °C protected from light. Before use, its contents in active ingredients was always determined. Margarine was coloured at $+40$°C, after careful melting and simultaneous introduction of nitrogen gas. Protected from light, the samples were frozen after being mixed with the colouring agent; cut into units required for the examinations and packed into aluminium foil lined with parchment, and kept at a temperature of ± 2 °C until used. Each of the enriched samples contained about 0.001% of β-carotene. Enriched with this concentration of β-carotene, margarine had a pleasant colour similar to that of summer butter; and its biological value was significant.

We have carried out preliminary examinations in order to find out the optimal concentration of tocopherols and synergents. Tocopherol concentrations

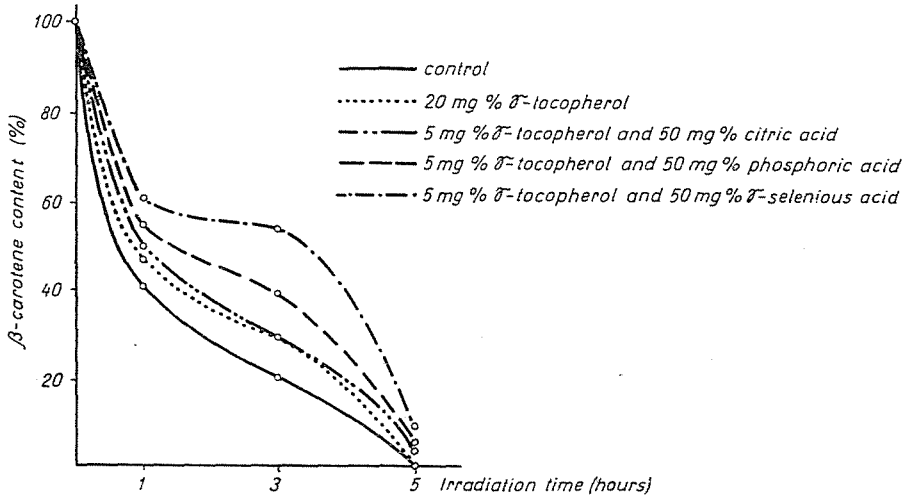

Fig. 3. Effect of γ-tocopherol on the β-carotene content of enriched margarin during UV irradiation

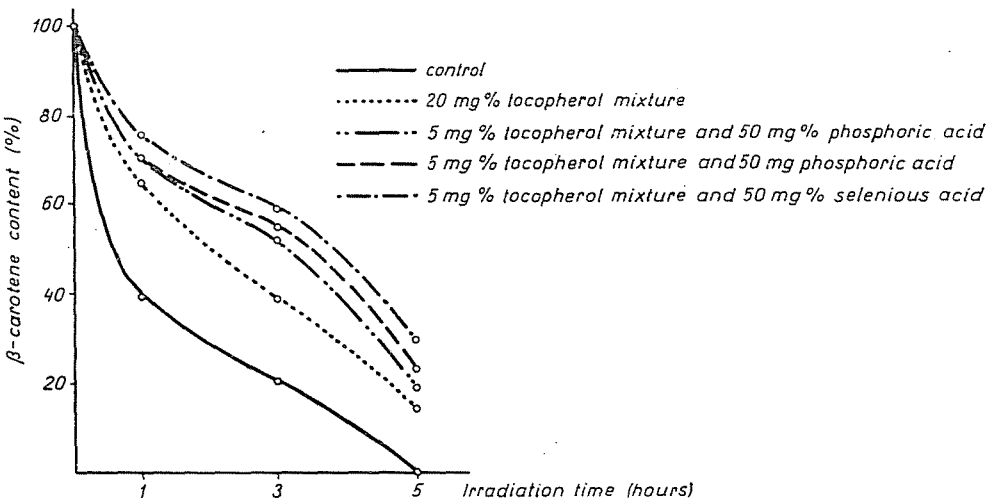

Fig. 4. Effect of tocopherol mixture on the β-carotene content of enriched margarin during UV irradiation

of 50, 20 and 10 mg% were used. In the presence of synergists a tocopherol concentration of 5 mg% was sufficient.

The necessary quantities of tocopherol and of synergists dissolved in absolute alcohol were mixed with butter in a nitrogen current, then the solvent was carefully eliminated in vacuum at a temperature of 40 °C. The prepared butter fat (further: butter) samples were irradiated or treated according

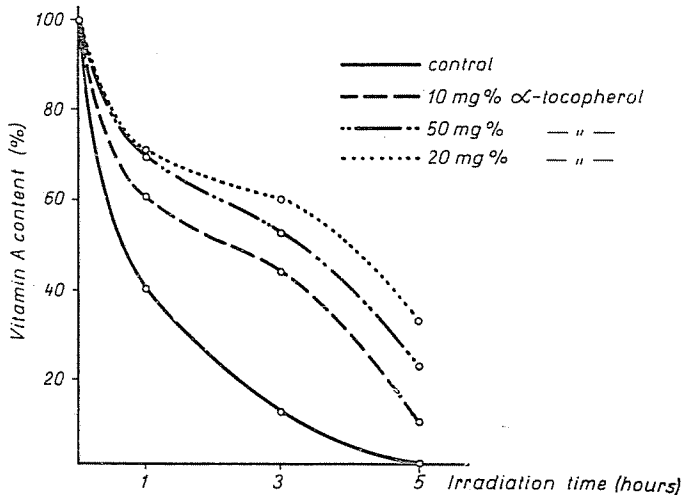

Fig. 5. Variation of vitamin-A content of butter stabilized with α-tocopherol during UV irradiation

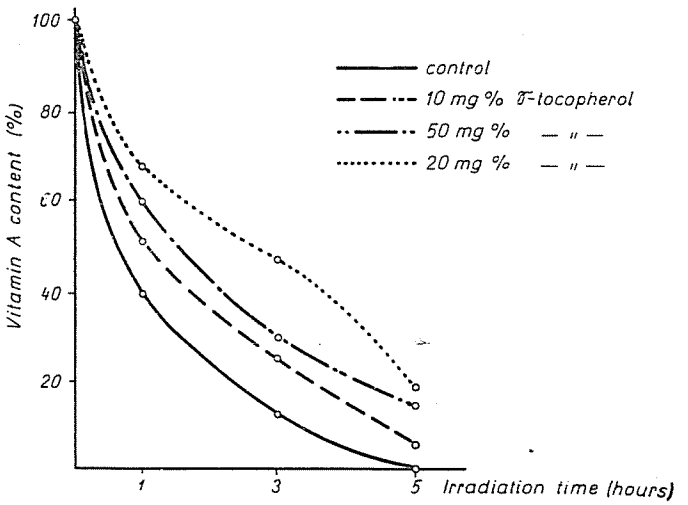

Fig. 6. Variation of vitamin-A content of butter stabilized with γ-tocopherol during UV irradiation

to the Active Oxygen Method. In both test substances the changes of β-carotene, peroxide and vitamin A content were controlled.

Table 1 shows the effect of tocopherols and synergists in margarine and butter enriched with β-carotene during ultra-violet irradiation;

Table 2. supplies informations under circumstances of AOM;

Fig. 1 shows in the presence of different synergists in enriched margarine the changes of β-carotene during ultra-violet irradiation; Figs 2—4 also

Table 2

Effect of tocopherols and acid synergents in enriched margarin and butter during AOM-treatment

Tocopherols	Conc. mg / 100 g	Acid synergents	Conc. mg / 100 g	AOM stability		Antioxidative index		% content of A-vitamin of butter		
				of margarin hours	of butter hours	in margarin	in butter	originally %	after 15 hours	at the end of AOM
—	—	—	—	4.00	42.00	1.00	1.00	100.00	—	—
	50.00			11.00	58.00	2.75	1.38		26.50	5.00
α	20.00	—	—	15.00	78.00	3.75	1.86	100.00	54.00	15.00
	10.00			9.00	45.00	2.25	1.04		12.00	—
	50.00			13.00	72.00	3.25	1.72		12.00	—
γ	20.00	—	—	18.00	88.00	4.50	2.10	100.00.	32.00	7.00
	10.00			11.00	50.00	2.75	1.19		5.00	—
	50.00			19.00	80.00	4.75	1.90		17.00	2.00
mixture	20.00	—	—	24.00	90.00	6.00	2.15	100.00	48.00	10.00
	10.00			14.00	62.00	3.50	1.47		9.00	—
		citric		22.00	79.00	5.50	1.88		55.00	15.00
α	5.00	phosphoric	50.00	26.00	82.00	6.25	1.95	100.00	57.00	16.00
		selenious		34.00	90.00	8.50	2.15		60.00	20.00
		citric		30.00	90.00	7.50	2.15		37.00	8.00
γ	5.00	phosphoric	50.00	38.00	97.00	9.50	2.30	100.00	39.00	9.50
		selenious		45.00	102.00	11.25	2.45		42.00	11.00
		citric		40.00	97.00	10.00	2.30		50.00	10.00
mixture	5.00	phosphoric	50.00	47.00	100.00	11.75	2.38	100.00	52.00	12.00
		selenious		56.00	110.00	14.00	2.61		55.00	16.00

show the changes of β-carotene content in enriched margarine during ultra-violet irradiation in the presence of α-tocopherol, γ-tocopherol, tocopherol mixture and synergists; Figs 5—6 show the alteration of vitamin A in the presence of different concentrations of α- and γ-tocopherol.

Summing up the results: In margarine and butter enriched with β-carotene the tocopherol concentration of 20 mg% proved to be the quantitative optimum for the prolongation of the induction period under circumstances of both ultra-violet irradiation and AOM, irrespective of the sort of tocopherol, while the qualitative optimum was achieved with a natural tocopherol mixture. During ultra-violet irradiation, synthetic tocopherol also displayed advantageous properties.

As a synergist (with 5 mg% of tocopherol mixture), selenious acid had the best effect.

During ultra-violet irradiation, the tocopherol mixture and a combination of tocopherol mixture selenious acid still preserved 30% of the original β-carotene content as in the fifth hour of experiments. If AOM was applied, β-carotene content was decomposed in spite of the same protection.

During irradiation in case of butter treated with AOM, the most efficient protection of vitamin A was exerted by α-tocopherol and, in the presence of synergists, by a combination of α-tocopherol and selenious acid; at the end of 5 hours of irradiation, 48% of initial vitamin A contents remained. This observation might explain the outstanding biological and physiological effects of α-tocopherol *in vivo* equally based on the protecting of vitamin A in the organism.

Summary

The most probable two explanations of the behaviour of tocopherols in reactions as derived from their chemical character are as follows:

1. As electron carriers tocopherols participate in the biological oxidation, in respiration and in other biological processes.

2. As intracellular antioxidants tocopherols protect unsaturated fats and similar oxygen-sensitive organic compounds, e.g. A-provitamines, A-vitamines, ascorbic acid, etc., against autooxidation.

From the point of view of food science and industry the most important property of tocopherols is their antioxidative activity which protects lipids and fat foods. As is known, the production of margarine enriched in A-provitamines is of utmost practical importance, however, this is practicable only if the added carotene remains unchanged during storage. Research work to find methods of protecting carotene in enriched margarines and A-vitamines in butter has been carried on. Synthetic and natural tocopherols have been used as stabilizers preventing oxidation in artificial ageing experiments.

Tocopherols were found to lengthen the induction periods, protect the A-vitamins and particularly the provitamines, and their chemical derivates did not negatively affect the organoleptic properties of the food.

References

1. TELEGDY-KOVÁTS, L.: Válogatott fejezetek az élelmiszerkémiából. Vitaminok. (Selected chapters of food chemistry, Vitamins). Tankönyvkiadó, Budapest, 1962.
2. HARRIS, R. S.—WOOL, I. G.—MARRIAN, G. F.—THIMANN, K. V.: Vitamins and Hormones. Academic Press, New York. London. **20** (1962).
3. BAUERFEIND, J. C.—ROKOSNY, A. D.—SIEMERS, G. F.: Food Eng. **25,** 81 (1953).
4. KOEHN, C. J.: Arch. Biochem. **17,** 337 (1948).
5. BURNS, M. J.—HAUGE, S. M.—QUACKENBUSH, F. W.: Arch. Biochem. **30,** 341 (1951).
6. ANGLIN, C.—MAHON, J. H.—CHAPMAN, R. A.: Dairy Science **38,** 333 (1955).
7. SCHWARZ, K.—FOLTZ, G. M.: Am. Chem. Soc. **79,** 3292 (1957).
8. BIERI, J. G.—ANDREWS, K. L.: J. Am. Oil Chem. Soc. **40,** 365, 1963.
9. LEA, C. H.: Rancidity in Edible Fats. Chem. Pub., New York, 1939.
10. BIEFER, K. W.: Mitt. Geb. Lebensm. u. Hyg. **53,** 243 (1962).
11. JACOBS, M. B.: The Chemical Analysis of Foods and Food Products. IIIrd edition. D. Van Nostrand Comp., New York, London, 1958.

12. MONIER-WILLIAMS, G. W : Trace Elements in Food, Chapman and Hall, London, 1949.
13. Co-operative of the Institute: Methodological Exercises in Food Industry (Élelmiszeripari metodikai gyakorlat). Tankönyvkiadó, Budapest, 1965.
14. MOOR, H.: Mitt. Geb. Lebensm. u. Hyg. **44**, 257 (1953).
15. LÁSZTITY, R.—M. SZILAS-KELEMEN—É. BERNDORFER KRASZNER—F. ÖRSI—J. VARGA: Élelmezési Ipar (Food Industry) **20**, 163 (1966).
16. LUCKMANN, F. N.—MELNICK, D.—MILLER, J. D.: J. Am. Oil Chem. Soc. **30**, 602 (1963).

Prof. Dr. László TELEGDY KOVÁTS
dr. Éva BERNDORFER KRASZNER
Budapest, XI. Műegyetem rkp. 3.
Hungary

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Farkas Sándor
A kézirat nyomdába érkezett: 1967. I. 16. — Terjedelem: 16,75 (A/5) ív, 117 ábra

67.63365 — Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György