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The use of antioxidants in the storage of cod liver oil (Paper No. 4 in collection of papers on "Technology of fats oils and food products")

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The use of antioxidants in the storage of cod liver oil

/50*

Cod liver oil, on storage, acquires an unpleasant taste and odour, since in comparison with other fish oils it contains many highly unsaturated fatty acids [12, 27] capable of being oxidized.

The oxidation products impart an unpleasant taste and odour to the oil and are, at the same time toxic [6, 7, 11, 23]. It is therefore necessary to find methods for the storage of cod liver oil which prevent or suppress its oxidation for a comparatively long period of time. This is especially important if we are to consider that this oil is used for medicinal purposes.

Numerous works have been written on the subject of the inhibition of oxidation in various fats and oils by the

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* Numbers in the right margin indicate the page numbers of the original.

use of antioxidants. Only a few of those antioxidants are effective in the shielding of fish oils from oxidation, in particular, cod liver oil [5, 19, 20]. Many research workers rate highly the following fat antioxidants: BHA (butylhydroxyanisol), BHT (butylhydroxytoluol), propyl-, ethyl-, dodecylgallates, and thioxane* [1, 3, 9, 15-17, 22, 24-27].

These reagents are authorized for use in the food industry in various countries. In the USSR, at present, only BHT and BHA are authorized for use.

In order to choose the most active antioxidants for cod liver oil (medicinal), it was necessary to test the antioxidative activity of several oxidizing agents.

We first established that the oil begins to oxidize as soon as it is melted down. It was therefore decided to conduct experiments with the introduction of an antioxidant at the very beginning of the melting down of the oil from the liver. It was necessary to find out how much antioxidant to introduce into the liver, in order that there should remain in the oil no more of this antioxidant than is permitted by the standards of the Ministry of Health of the USSR.

We also had to establish the ideal times for the storage of cod liver oil in the presence of antioxidants.

* An antioxidant, patented by France in 1958; its active ingredient is ascorbic acid.

The determination of the antioxidative activity of butylhydroxyanisol (BHA), butylhydroxytoluol (BHT) and thioxane. The activity of individual antioxidants was determined by establishing the period of induction of /51 samples of oil with given antioxidants at a temperature of 40°C. For this, the samples of high grade oil, taken from the cod liver by melting down under conditions of production, were placed in duplicate in a weighing bottle, where a specific quantity of antioxidant was introduced. Two tests were set for each aliquot of antioxidant. As a control, there were two weighing bottles of oil without antioxidant.

The samples of fat were kept at a thermostatically controlled temperature until there was an accumulation of peroxide compounds in a quantity corresponding to a peroxide number of 0.1% iodine.

On the basis of the results obtained, the times for the induction of the oil with the tested antioxidants were established. Generally, the time (in hours) during which the peroxide number of the oil reaches the value of 0.1%, is taken as the period of induction.

For comparison, the period of induction of the oil was determined for values of the peroxide numbers of the oil corresponding to 0.05; 0.07% iodine (Table 1)*.

* Tables at the end of translation. Translator.

The relative effictiveness of the antioxidants is determined as a ratio of the rate of oxidation of the oil with antioxidant, to the rate of oxidation without antioxidant.

The results obtained on the activity of BHA differ from our previous findings [12], according to which the activity of BHA, at the usual temperature of the storage of oil, is higher than the antioxidative activity of BHT. At the same time, they agree with the findings of Japanese research workers [24], who tested the activity of BHA in the oxidation of several fatty acids at a temperature of 97.8°C.

Such discrepancies in the evaluation of the activity of BHA and BHT, evidently, are explained by the instability of BHA at elevated temperatures; this indicates the unsuitability of the use of BHA in the melting down of oil from liver [21].

For the determination of the quantity of antioxidant which disintegrates when the fat is melted down, and remains in it, sterilized liver of Atlantic cod was used.

The fat from the liver was melted down in a glass flask, which was heated in a boiling water bath. Into the flask, through a glass tube, was fed live steam from a steam generator, simulating the technological process of obtaining oil on fishing trawlers.

For the experiment, 300 grams of cod liver were taken, into which a given quantity of antioxidant was introduced.

After the thermal treatment of the liver, the mass /52 which had not completely congealed, was separated as completely as possible in a Büchner funnel. The oil was separated from the liquid fraction in a separatory funnel and weighed.

The yield of oil generally constituted 120-130 grams; that is, 40-45% of the liver by weight. Before analysis, the oil was dehydrated with sodium sulphate, and filtered. The quantity of BHA retained in this oil was determined by a method based on a colour reaction of BHA with 2, 6-dichlorquinonechlorimide extracted from the oil with 72% ethanol [10].

BHT was separated from the oil by distillation with superheated steam, and its percentage in the distillate was determined by a colour reaction with ferric chloride and α, α_1-dipyridine.

The results obtained in this case showed that 50-70% of the antioxidant introduced into the liver appears in the oil (Table 2).

The experiments which were carried out confirmed our hypothesis that when an antioxidant is introduced into the liver itself, the oil upon melting down is changed to a lesser degree than when the oil is melted down without antioxidants.

Production and storage of oil from the liver of Baltic cod. For the research, oil was used which was especially melted out at the fishing enterprise at Liepaja. The liver was extracted from the cod and delivered from the fishery in a refrigerated state. As a rule, the fish are not dressed immediately; therefore, the quality of the liver, as a result of enzymatic hydrolysis, was not high, and the oil obtained from this liver was not always good. Two batches of oil had high acid numbers (2, 8 and 3, 6), although other properties characteristic of a degree of oxidation of the oil (peroxide number, carbonyl number, retention of oxiranic oxygen) were low.

The characteristics of the liver of Baltic cod, from which the oil was obtained for experimental storage, are presented in Table 3.

The oil was melted down from the liver according to the standard processing at the Liepaja fishery. At the beginning, the liver is heated in a kettle with steam, then the oil is melted down by means of a supply of live steam into the kettle. /53

The melting down was carried on for 2.5 hours at a temperature of the liver of 105-110°C. After this, the steam inlet into the kettle was cut off, and the contents were allowed to settle for 40-60 minutes. Then the oil was decanted into a vessel in which it was again allowed to settle, this time for 1-1½ hours. The settled oil was then decanted into a flask. In our tests, the oil was decanted into milk cans and stored at a temperature around 15°C.

Seven tests were performed. In two tests, before the melting out of the oil, 0.03% BHT was introduced into one liver sample, and 0.002% propylgallate was introduced into another. In another two tests, antioxidants were introduced into the melted oil; in one case, 0.2% BHT was used, and in the other, 0.001% propylgallate. In two tests, the oil was melted down without antioxidant. Simultaneously, 0.2% citric acid (of liver, or of oil, by weight) was used.

A study of the composition of highly unsaturated fatty acids from cod liver showed the oil from the liver of Baltic cod retains more of the highly unsaturated fatty acids than does the liver of Atlantic cod [12]. The amount of these acids in the oil of Baltic cod approaches 39%, while the acids with five double bonds constitute 15-21% (Table 4).

Periodically, during the storage of the oil, average samples were taken at all levels. In these samples, the degree of oxidation was determined, as well as the organoleptic properties. From the results obtained, which are presented in /54

Tables 5 and 6, it is evident that the oil, separated by melting with the use of antioxidants, is better than the oil melted down without them, in which there were more peroxide compounds. However, upon storage, the oils melted out with antioxidants, and the oils in which the antioxidants were added after melting, showed similar changes. Two samples (Numbers 15 and 16) are an exception; their acid numbers were high at the very beginning of the storage period. These oils, on an organoleptic basis, were removed from storage after 80 days.

The samples of oil stored without antioxidants changed to a greater extent and more quickly. This is evident from the values of their peroxide number, and the amount of oxiranic oxygen, as well as their organoleptic analysis. Sample Number 12 appeared to be the best of the seven samples of oil from the Baltic cod. This sample, in the presence of 0.02% BHT, was stored for 80 days without signs of oxidation.

In addition to this, it follows from these results that the oil from the liver of Baltic cod is oxidized at the same rate as the oil from the liver of Atlantic cod, which retains significantly less tocopherol (12-15 mg %) [12]. Evidently, the highly unsaturated fatty acids of this oil form unstable peroxides which do not have a chance to react with the tocopherol. On account of this, the oil is greatly oxidized

in spite of the high retention of tocopherol (vitamin E). The amount of Vitamin A in the oil changes insignificantly, or not at all.

Production and storage of oil from the liver of Atlantic cod. Two sets of tests were carried out. The first series of five tests was done on a fishing trawler anchored at the port of Murmansk. The liver used was delivered to the port in a refrigerated state. The quality of the liver was good as seen from the results in Table 7.

The oil was obtained by the method used on the fishing trawlers [14].

In two tests, when the oil was melted down, 0.01% BHT and 0.001% propylgallate (of liver, by weight) were added; in another two, 0.015% BHT and 0.001% propylgallate, by weight of oil obtained by melting down.

One test was a control.

The oil in these tests retained more highly unsaturated acids (Table 8) than the same oil which we tested earlier [12]. Evidently, this is due to biological variations of the cod, caught at various times of the year and in various regions.

L.C. Losev, a scientific worker of PINRO on the fishing trawler carried out the second series of tests (Numbers 6-10)

(see Table 7). He performed two tests with antioxidant introduced into the liver before the oil was melted out of it (0.01% BHT and 0.001% propylgallate). He performed two other tests with antioxidant added to the melted oil (0.015% BHT and 0.001% propylgallate); one test was a control.

The oil, obtained from these tests, was poured into milk cans and stored for a month on a ship, in the same place as the oils for tests 1-5, at a temperature of approximately 15°C.

The oil in test 4 to which 0.015% butylhydroxytoluol was added, fared better in storage than the others, with the exception of the oil of test 9 where the same antioxidant was added to the liver before the oil was melted from it (Tables 9 and 10). The first sample of oil retained a good odour /56 after 120 days, and the second - after 105 days of storage; the other samples of oil acquired an odour of oxidized oil in a very much shorter time.

Propylgallate in an approved concentration prevents the oil from oxidation for longer periods than does BHT. However, when propylgallate is introduced into the liver before melting, it imparts to the oil an extraneous odour, similar to that of melted butter. It is possible that this is the result of an incomplete breakdown of the propylgallate at elevated temperatures. Later on it became clear that the

imported propylgallate which we used was an impure variety, /58 and its activity was 1.5 times lower than that of the purified propylgallate. For this reason, the amount of propylgallate shown in Table 9 is 1.5 times too high in comparison with the true value.

When cod liver oil is stored for long periods, the peroxide number and amount of oxiranic oxygen do not always increase; sometimes their values temporarily decrease, then increase. This results in the conversion of peroxide and epoxy compounds to products which are more highly oxidized. Therefore, each of these properties, taken individually, does not always sufficiently characterize the degree of oxidation of the oil at given stages of its storage. The carbonyl number of cod liver oil remains nearly unchanged.

In the meantime, the problem of choosing the most characteristic, unbiased property of the degree of oxidation of cod liver oil, is unsolved.

In spite of this, we have interesting results on the values of the peroxide numbers of samples of cod liver oil, in which clear signs of oxidation were found.

Antioxidant and its Concentration, %	Length of Storage, in days	Peroxide number of the oil, % iodine
Without antioxidant	60	0,11
	75	0,1
BHT, 0.01% of liver, by weight	120	0,1
	135	0,13
Propylgallate, 0.001% of liver, by weight	150	0,18
	150	0,16
BHT, 0.15% of oil, by weight	150	0,17
	135	0,11
Propylgallate, 0.001% of oil, by weight	150	0,09
	150	0,10

Retention of residue*. The residue remaining after /59 the oil is melted down from the liver contains the following, in relation to the total of the obtained oil: moisture - 47-59%, oil - up to 38%, protein - up to 15%.

Depending on the freshness of the liver from which the oil is obtained, and the sanitation conditions of production, the residue can be used as a food product.

Generally, the residue is used as a feed for animals. Sometimes, when there are no provisions on the trawler for its storage, the residue is not used.

In the oil of the residue under the influence of enzymes and the oxygen of the air, peroxides, aldehydes, hydroxy acids, as well as free fatty acids are formed; the residue acquires the odour of oxidized oil.

* ? "Graksa" in Russian. Translator.

Since several products of the oxidation of the oil are toxic, we had to find a way not only of preserving the residue from decay, but also of preventing the oxidation of the oil.

For this purpose, several tests were carried out on the storage of the residue under laboratory conditions.

Here, the same change was observed in the residue as that which had been produced in the tests on the separation of the oil from the liver in the presence of the antioxidants BHA and BHT; in several tests, the antioxidant was added directly to the residue.

As a preservative, sodium chloride was used (3-10% of the residue, by weight); the residue was stored in glass jars filled to capacity (100-150 grams at 20-25°C).

The results of these tests are presented in Table 11. In the residue, to which 8 and 10% sodium chloride had been added, mold appeared in 35-40 days, but the putrid odour was absent.

There was no odour of oxidized oil in the residue with antioxidants after a period of 20-30 days.

The values of the characteristics of oxidative decay of the oil in the residue with antioxidants show that the

process of the oxidation of the oil is retarded, and it is possible to store the residue for 20-30 days without the aforementioned signs of oxidation.

The residue was stored for a longer time with sodium chloride as a preservative, and with 0.01% BHT or BHA, without the appearance of mold.

CONCLUSIONS

1. It was confirmed that cod liver oil is easily oxidized. This necessitates the search for means of prevention or inhibition of the oxidation. /67

2. BHT, in a concentration of 0.015-0.02%, was the most active of the antioxidants tested (BHT, BHA, thioxane, propylgallate) for cod liver oil. In oil melted down with the use of BHT, fewer oxidation products were found than in the oil melted down without antioxidants. The addition of antioxidant when the oil is melted down does not lengthen the period of its storage in comparison with the addition of antioxidant after the melting down.

3. The addition of 0.002% BHT by weight of oil of Atlantic cod lengthens its storage time up to 3-4 months without obvious signs of oxidation. The same amount of BHT lengthens the period of storage of Baltic cod liver oil up

to 3 months. Since the All-Union State Standard specifies that cod liver oil be stored for a year, it is necessary to continue the search for ways to guard the medicinal oil from oxidative decay.

4. There is no single property of oxidative decay that clearly defines the degree of oxidation of cod liver oil. However, cod liver oil with a peroxide number equal to 0.2, as a rule, is oxidized.

5. According to the laboratory results of the tests, residue with a retention of 45-60% of water and a concentration of 8% sodium chloride keeps well in storage for months at a temperature of approximately 20°C. Lower temperatures lengthen the storage period of the residue. The introduction into the residue of 0.02% by its weight of BHT prevents the oil from oxidation. It would be advisable to perform similar tests under conditions of production.

BIBLIOGRAPHY /67

1. Asnina, F.I. Novye antiokisliteli i emulgatory dla tipovykh zhirov. (New antioxodants and emulsifiers for standard fats). Trudy Ukrainskogo nauchno-issledovatel'skogo instituta pishchevoi promyshlennosti. (Trudy, Ukrainian Scientific Research Institute for the Food Industry, Issue 2, 1959).

2. Bukhman, N.D. Vliyanie antiokislitel'a-propilgallata na pishchevuyu tsennost' zhira. (The effect of propylgallate as an antioxidant on the food value of fat). Trudy 2-i konferentsii po voprosu "Problema zhira v pitanii". Trudy, 2nd Conference on the Question "The Problem of Fat in Food". Leningrad, 1962.

3. Gerchuk, M.P., Ivanova, V.M. Primenenie khimicheskikh preparatov dla zashchity pishchevykh zhirov ot progorkaniya. (The use of chemical compounds for the protection of food fats from rancidity). Pishchepromizdat, 1958.

4. Golland, B.A., Petrov, N.A. Povyshenie stoykosti zhirov i zhirosoderzhashchikh productov. (Increased stability of fats and fat products). Pishchepromizdat, 1958.

5. Davidova, Yu. C., Treshcheva, V.I. Primenenie okislitelei pri khranenii gidrozhira i treskovogo zhira.

(Use of antioxidants in the storage of hydrogenated fats and cod liver oil). Rybnoe khozyaistvo, 1958, No. 10.

6. Davyadorzh, D. Biologicheskie opyty po izucheniyu toksichnosti zhira, khranivshegos'a v raznykh usloviyakh. (Biological experiments on the study of toxicity in fats stored under various conditions). Trudy 2-i konferentsii "Problema zhira v pitanii". (Trudy, 2nd Conference on "The Problem of Fat in Food". Leningrad, 1962).

7. Kadykov, B.I. Nekotorye aktualnye voprosy v probleme "Zhir kak faktor pitaniya". (Several urgent questions on the problem: Fat as a factor in food). Trudy 2-i nauchnoi konferentsii "Problema zhira v pitanii". (Trudy, 2nd Scientific Conference on "The Problem of Fat in Food". Leningrad, 1962).

8. Karplyuk, I.A. Vliyanie fenolnykh antiokislitelei pishchevykh zhirov na zhivotnyi organism i ikh gigienicheskaya otsenka. (The effect of phenolic antioxidants of food fats on the animal organism and their hygienic evaluation). Trudy 2-i konferentsii "Problema zhira v pitanii". (Trudy, 2nd Conference on "The Problem of Fat in Food". Leningrad, 1962).

9. Lebedeva, V.K. Povyshenie stoikosti rafinirovannykh rastitelnykh masel pri pomoshchi antioksidantov. (Increased stability of purified plant oils with the help of antioxidants). Masloboino-zhirovaya promyshlennost' (Butter and Fats Industry), 1959, Number 7.

10. Krylova, N.N., Lyaskovskaya, Yu. S. Fiziko-khimicheskie metody issledovaniya produktov zhivotnogo proiskhozhdeniya (Physico-chemical methods of investigation of products of animal origin). Pishchepromizdat, 1961.

11. Parteshko, V.G. Ob okislitelnoi porche meditsinskogo rybnogo zhira. (On the oxidative decay of medicinal fish oil). Trudy 2-i konferentsii "Problema zhira v pitanii". (Trudy, 2nd Conference on "The Problem of Fat in Food). Leningrad, 1962.

12. Perepletchik, R.R., Davydova, Yu.S., Novikova, E.I. Soderzhanie vysokonepredelnykh zhirnykh kislot v treskovom zhire. (Retention of highly unsaturated fatty acids in cod liver oil). Trudy, VNIRO. Vol. XLV, 1962.

13. Perepletchik, R.R., Korduban, T.A., Maksimov, S.I. /68
Usovershenstvovanie tekhnologii proizvodstva i uslovii khraneniya zhira iz pecheni treski. (An advance in the technology of the production of cod liver oil and of the conditions of its storage).

Annotatsiya nauchnykh rabot VNIRO po planu 1962 g. (Annotation of scientific works of VNIRO on the 1962 project. Moscow 1963).

14. Sobolev, N.K., Bulashevich, M.T. Meditsinskii treskovyi zhir. (Medicinal cod liver oil). Murmansk, 1952.

15. Udlinenie srokov khraneniya pishchevykh zhirov. (Prolongation of the storage period of food fats and oils). Sbornik nauchnykh rabot, vypolnennykh VNIIMP, b 1957 g. Pishchepromprodukt, 1958. (Symposium of references of scientific works compiled by VNIIMP (All-Union Scientific Research Institute of the Dairy Industry) in 1957. Food Industry Products, 1958.

16. Emanuel' N., Knorre D., Lyaskovskaya, Yu., Piul'skaya, V. Primenenie butiloksianizola dla povysheniya stoikosti pishchevykh zhirov. (The use of butyloxyanisol for increasing the stability of food fats and oils). "Myasnaya industriya SSSR" (Meat Industry of the USSR), 1955, No. 6.

17. Emanuel' N., Knorre, D., Lyaskovskaya, Yu., Piul'skaya, V. Antioxisliteli dla povysheniya stoikosti zhivotnykh zhirov. (The use of antioxidants to increase the stability of animal fats). "Myasnaya industriya SSSR".

("Meat Industry of the USSR", 1958, No. 2).

18. Emanuel', N.M. Lyaskovskaya, Yu.N. Tormozhenie protsessov okisleniya zhirova. (Inhibition of the processes of oxidation of fats and oils). Pishchepromizdat, 1961.

*19. Chanina M.H., El-Shobazi F.A., stability of oils and fatty foods. I. A comparative evaluation of several untioxidants in shark liver mexhyl esters. Grasas y aceites, No. 2, 14, 1963.

+ *20. Hiljemark A. Aas H.W., Marcus R. Verbesserung der Haltbarke frische, fetter. Fische durch Behandlung mit Antioxydantien. Fette- Seifen- Anstrichmittel, No. 6, 61, 1959.

*21. Sims Rex Y., Hilfman Lee. Stabilization of edible animal fats during rendering. J. Amer. Oil Chem. Soc., No. 9, 33, 1959.

*22. Stuckey B.W. Antioxidants in food products. Canad. Food Ind. No. 10, 31, 1960.

*23. Takashi Kaneda, Hisal Sakai. Nutritive value or toxicity of highly unsaturated fatty acids II. J. Biochem. 42, 5, 1955.

* Transcribed unaltered. Translator.

+ For translation see p.21a

24. Tajama Kenzo. Reaction mechanism for antioxidants applied to marine products. I. Relation between the efficancy of antioxidant and the degree of unsaturation of substrate oil. J. Tokyo Univ. Fish., No. 1, 48, 1962. Ekspress Infori. VINITI, Rapid Information (VINITI, All-Union Institute of Scientific and Technical Information), Fish Industry, No. 3, 1963.

+*25. Troellich R., Öl und Fettantioxydation und Antioxydantien in Spegel der Literatur, Pharmazie, N 1, 18, 1963.

+*26. Trollenaar F.D., Voc H.J. Untersuchungen über die Verwendung von butylirtem Hydroxytoluol als Antioxydans in Lebensmittel. Fette-Seufen-Anstrichmittel N 2, 58, 1956.

27. Tsuyuki, Khideo. Zhiry morskikh zhivotnych i ryb i protovookisliteli zhirov. (Fats of sea animals and fish and their antioxidants). "Sui Sankai", No. 919, 1961. Extracted from the reference journal Chemistry, 1962, 14N377, 17N**284.

+*28. Well R. Emploi des antioxygenes dans lälimentation le cas du tioxan. Oleagineux, 1960, 15.

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* Transcribed unaltered. Translator.
** Could be "H". Translator.
\+ For translation see p.21a.

+ Translation of German and French titles in bibliography:

20. Improving the stability of fresh fat fish by treatment with antioxidants.

25. Oil and fat antioxidation and antioxidants in literature.

26. Studies on the use of butylated hydroxytoluene as antioxidant of foods.

28. The use of antioxidants in foods; the case of tioxane.

Table 1 /51

Antioxidative activity of several antioxidants

Antioxidant and its concentration in the oil, in %	Time of induction (in hours) to the value of the peroxide number			Relative effectiveness, E
	0.05	0.07	0.10	
Control	11.8	14.5	19	-
BHA 0.05 + citric acid 0.1	12.5	16.8	23	1.14
BHT 0.05 + citric acid 0.1	23.0	27.0	29	1.78
Thioxane 0.05	12.0	17.0	23	1.13

Table 2 /52

The change in quality of the oil in the process of the heat treatment of the liver (laboratory experiments)

Experiment number	Antioxidant	Antioxidant added to the liver, % of weight of its mass	Appearance in the oil after heat treatment, % of weight of the oil	Peroxide number of the oil, % iodine		Retention of oxiranic oxygen, mg %		Carbonyl number mg. KOH in 1 gm.		Acid number mg. KOH in 1 gm.		Vitamin A (I.U.) per gram			
												liver		oil	
				I	II	I	II	I	II	I	II	I	II	I	II
1	BHT	0,02	0,029	0,0038	0,0048	—	4,9	0	1,2	0,8	0,8	120	120	350	350
2	BHT	0,02	0,026	0,0014	0,002	1,5	4,7	0	1,8	—	—	120	115	300	320
3	BHA	0,03	0,028	0,0014	0,007	—	—	—	—	0,7	0,7	140	150	370	330
4	BHT	0,03	0,033	0,0013	0,007	1,5	2,8	0	0,6	0,7	0,7	150	140	370	370
5	BHA	0,02	0,030	0,0013	0,008	1,4	2,5	0	0,7	0,4	0,4	200	160	350	350
6	BHT	0,03	0,012	0,004	0,01	1,4	4,9	0	0,7	0,4	0,5	—	—	—	—
7	BHT	0,02	0,026	0,00	0,007	1,5	4,1	0	2,2	0,4	0,7	120	115	280	270
8	—	—	—	0,0038	0,013	1,4	5,9	0	2,2	0,5	0,7	140	140	350	350
9	—	—	—	0,0014	0,026	1,4	6,1	0	3,1	0,5	0,7	160	170	320	340

Note: I - before heat treatment

II - after heat treatment

Table 3. /53

The chemical composition of the liver of Baltic cod and the quality of the oil separated from it

Experiment number	Chemical composition of the liver, %			Properties of the liver oil						Retention of vitamins in the liver	
	Moisture	Oil	Total protein	Peroxide number % iodine	Retention of oxiranic oxygen mg. %	Carbonyl number	Thiobarbituric number	Acid number		A (I.U.) per gram	E, mg. %
11,12	40,8	44,8	14,4	0	1,7	0	1,1	0,4		290	45
13,14	43,6	45,5	10,9	0	1,7	0	1,3	0,6		370	45
15	41,0	47,7	11,3	0,0016	1,5	0	1,4	0,9		300	60
16	34,4	57,1	8,5	0,0011	1,4	0	1,3	0,9		500	60
17	42,0	47,8	10,2	0,0024	1,5	0	1,1	1,8		460	45

Table 4.

Composition of highly unsaturated fatty acids from the liver of Baltic cod, %

Sample number of the oil	Antioxidant and its concentration	With two double bonds (linolenic type)	With three double bonds (linolenic type)	With four double bonds (arachidonic type)	With five double bonds (clupanodonic type)	Total highly unsaturated fatty acids
11	Control	6,4	5,5	4,1	20,4	36,5
12	BHT, 0.02% of oil, by weight	6,14	5,2	4,0	18,8	34,2
13	Control	6,3	4,0	3,5	15,4	29,1
14	Propylgallate, 0.001% of oil, by weight	7,4	5,6	3,7	17,2	34,0
15	BHT, 0.03% of liver, by weight	7,9	6,6	3,6	20,8	39,0
16	BHT, 0.03% of liver, by weight	7,3	5,6	3,3	16,7	33,0
17	Propylgallate, 0.02% of liver, by weight	8,6	5,5	3,5	18,1	36,8

Table 5. /55

Changes in characteristics upon storage of oil derived from Baltic Cod

Characteristics of the oil	Test number, antioxidant, and its concentration						
	11 control	12; BHT 0.02% of oil by weight	13 control	14 propyl-gallate 0.001% of oil by weight	15 and 16; BHT, 0.03% of liver, by weight		17; propyl-gallate 0.002% of liver by weight
	At the beginning of storage period						
Peroxide number, % iodine	0,053	0,041	0,041	0,061	0,009	0,0011	0,022
Retention of oxiranic oxygen, mg. %	2,9	3,1	2,5	2,8	2,2	2,5	3,8
Carbonyl number	0	0	0,7	0,7	0,6	0,6	0
Acid number	0,6	0,8	1,2	1,6	3,6	2,8	1,7
Thiobarbituric number	1,1	2,4	1,8	1,4	1,6	1,6	2-
	After 20 days of storage						
Retention of vitamin A (I.U.) per gram	720	1000	1100	1190	840	950	860
Retention of Vitamin E, mg. %	116	116	130	120	145	141	110
Peroxide number	0,11	0,11	0,12	0,12	—	—	—
Retention of oxiranic oxygen, mg. %	8,2	7,8	7,1	7,5	—	—	—
Carbonyl number	1,2	1,3	1,4	1,3	—	—	—
Acid number	0,8	1,1	1,5	1,4	—	—	—
Thiobarbituric number	3,5	2,6	3,4	3,5	—	—	—
	After 50 days of storage						
Peroxide number	0,19	0,17	0,2	0,15	0,14	0,11	0,17
Retention of oxiranic oxygen, mg. %	5,9	4,6	5,3	4,5	4,1	3,9	4,1
Carbonyl number	2,6	2,6	5,2	3,3	2,6	1,6	1,7
Acid number	0,9	0,8	1,6	1,6	—	—	—
Thiobarbituric number	2,4	1,7	1,7	2,4	3,5	3,1	4,4

Table 5 (cont'd)

Characteristics of the oil	Test number, antioxidant, and its concentration					
	11 control	12; BHT 0.02% of oil by weight	13 control	14 propyl-gallate 0.001% of oil by weight	15 and 16; BHT, 0.03% of liver, by weight	17 propyl-gallate 0.002% of liver by weight
After 80 days of storage						
Retention of vitamin A, (I.U.) per gram	820	920	1100	1000	830	800	800
Retention of Vitamin E, mg. %	115	120	115	131	—	—	110
Peroxide number	0,28	0,19	0,20	0,15	0,16	0,15	0,26
Retention of oxiranic oxygen, mg. %	9,1	7,2	14,5	10,7	5,4	4,7	4,6
Carbonyl number	3,4	2,7	3,6	2,8	2,6	2,9	3,4
Acid number	1,1	0,8	1,8	1,3	3,0	2,7	1,9
Thiobarbituric number	3,1	2,4	2,1	1,5	1,9	2,5	2,1
After 100 days of storage							
Retention of vitamin A, I.U. per gram	720	950	1100	1150	850	900	850
Peroxide number	0,3	0,24	0,39	0,24	—	—	0,29
Retention of oxiranic oxygen, mg. %	3,3	3	4,3	3,65	—	—	4
Carbonyl number	2,4	2	3,2	2,6	—	—	2,4
Acid number	1,1	0,95	1,7	1,5	—	—	1,5
Thiobarbituric number	3	2,9	3	2,7	—	—	3,8
After 140 days of storage							
Retention of vitamin A, I.U. per gram	800	950	1150	1000	—	—	800
Peroxide number	0,7	0,58	0,79	0,58	—	—	0,65
Retention of oxiranic oxygen, mg. %	3,7	3,5	4,1	3,7	—	—	4,5
Carbonyl number	8,7	9	8,6	8,7	—	—	6,6
Acid number	1,4	1,1	1,7	1,5	—	—	1,8
Thiobarbituric number	5,1	3	4	4,2	—	—	4,2

Table 6

Changes in organoleptic properties of oil from Baltic cod, upon storage

Test number	Antioxidant and its concentration	50 days	80 days	100 days	140 days
11	Without antioxidant (control)	Odour of slightly oxidized oil	Odour of oxidized oil	Odour of oxidized oil	Odour of oxidized oil
12	BHT, 0.02% of oil, by weight	Normal odour characteristic of cod liver oil	No odour of oxidized oil	Slight odour of oxidized oil	Same
13	Without antioxidant (control)	Odour of slightly oxidized oil	Odour of oxidized oil	Odour of oxidized oil	Same
14	Propylgallate, 0.001% of oil, by weight	Same	Weak odour of oxidized oil, foreign odour	Same	Same
15	BHT, 0.03% of liver, by weight	Weak odour of oxidized oil	Odour of oxidized oil, foreign odour	Removed from storage	-
16	BHT, 0.03% of liver, by weight	Odour of slightly oxidized oil	Same	Same	-
17	Propylgallate, 0.002% of liver, by weight	Same	Odour of oxidized oil	Odour of oxidized oil	Odour of oxidized oil

Table 7 /58

Chemical composition of liver of Atlantic cod and the quality of its oil

Test number	Chemical composition of liver, %			Properties of the oil from the liver					Retention of Vitamin A, I.U. per gram
	Moisture content	Oil	Total protein	Peroxide number, % iodine	Retention of oxiranic oxygen, mg. %	Carbonyl number	Thiobarbituric number	Acid number	
1	40,8	44,8	7,1	0	2,0	0	1,09	0,16	670
2	42,7	47,1	10,2	0	1,9	0	1,15	0,15	450
3, 4, 5	40,5	46,6	12,9	0	1,5	0	1,15	0,15	470
6, 7	29,3	60,7	10,0	0	1,5	0	1,1	0,1	180
8, 9	29,2	59,5	11,3	0	2,1	0	1,1	0,2	180
10	28,9	59,8	11,3	0	1,9	0	1,1	0,2	190

Table 8 /59

Structure of highly unsaturated fatty acids in the oil of Atlantic cod liver (in %)

Sample number of the oil	With two double bonds (linoleic type)	With three double bonds (linolenic type)	With four double bonds (arachidonic type)	With five double bonds (clupanodonic type	Total highly unsaturated fatty acids
1	5,9	4,7	5,0	17,3	32,9
2	3,7	4,4	5,3	19,7	33,1
3	4,5	4,2	6,0	18,3	33,0
4	4,3	4,2	6,1	19,3	33,9
5	4,2	4,3	6,0	18,4	32,9

Table 9

Changes in the oil from the liver of Atlantic cod, upon storage

Properties of the oil	Test number, antioxidant and its concentration									
	1; BHT, 0.01% of liver by weight*	2; propylgallate, 0.01% of liver by weight**	3 Control	4; BHT 0.015% of oil by weight	5; propylgallate, 0.001% of oil by weight	6 Control	7; BHT 0.015% of oil by weight	8 and 9; BHT 0.01% of the liver by weight	10; propylgallate 0.001% of liver by weight
	at the beginning of the storage period									
Peroxide number, % iodine	0,062	0,053	0,053	0,04	0,044	0,025	0,014	0,021	0,02	0,032
Retention of oxiranic oxygen, mg. %	3,2	2,5	3,2	3,1	2,4	2,3	2	2,5	2,2	1,8
Carbonyl number	1,4	1,3	1,2	1,3	1,3	0	0	0	0	0
Thiobarbituric number	1,6	1,7	2,16	2,1	1,9	1,65	1,33	1,05	1,3	1,8
Acid number	0,3	0,3	0,7	0,4	0,5	0,7	0,54	0,94	0,43	0,8
Retention of Vitamin A (I.U. per gram)	600	650	730	700	750	650	650	650	650	650
	30 days					45 days				
Peroxide number % iodine	0,065	0,069	0,21	0,115	0,074	0,19	0,16	0,12	0,11	0,097
Retention of oxiranic oxygen, mg. %	3,4	2,6	3,8	2,5	2,4	3,3	2,7	3	2	2,3
Carbonyl number	1,5	1,4	1,4	1,5	1,7	2,5	1,8	2,1	2	2
Thiobarbituric number	2,5	2,9	4,6	2,4	4,9	6	6,2	5,1	4,2	4,1
Acid number	0,6	1,5	1,8	0,9	1,4	0,9	0,6	1,2	0,6	0,8
Retention of Vitamin A, I.U. per gram	600	650	750	700	750	610	630	650	640	640
	60 days					75 days				

Table 9 (cont'd)

Properties of the oil	1; BHT, 0.01% of liver by weight*	2; propylgallate, 0.01% of liver by weight**	3 Control	4; BHT 0.015% of oil by weight	5; propylgallate, 0.001% of oil by weight	6 Control	7; BHT 0.015% of oil by weight	8 and 9; BHT 0.01% of the liver by weight	10; propylgallate 0.001% of liver by weight
Peroxide number, % iodine	0,089	0,099	0,11	0,087	0,087	0,1	0,1	0,12	0,062	0,071
Retention of oxiranic oxygen, mg. %										
Carbonyl number	1,85	2	2,6	1,2	2,2	1,9	1,8	1,35	1,65	2
Thiobarbituric number	1,7	1,6	2,4	1,6	1,5	2,5	2,5	1,3	1,3	13
Acid number	3,8	2,9	2,8	2,5	3,4	2,4	2	3,4	4	3,7
Retention of Vitamin A, I.U. per gram	0,5	1,7	1,5	1,1	1,5	1	0,3	1	0,5	0,9
	600	650	750	700	770	680	580	580	590	580
			90					105		
Peroxide number, % iodine	0,07	0,11	0,065	0,06	0,08	0,081	0,12	0,10	0,14	0,066
Retention of oxiranic oxygen, mg. %										
Carbonyl number	2,1	3,1	1,9	1,8	1,5	2,7	2,5	1,8	2	2
Thiobarbituric number	1,3	1,2	2,1	1,2	1,1	3,7	3,2	3	3,1	2,4
Acid number	2	1	1,7	1,3	1,2	1,5	3,5	2	2,8	2,2
Retention of Vitamin A, I.U. per gram	0,7	2	2,2	1,1	1,5	1,3	0,7	1,4	0,6	1,26
	—	—	—	—	—	680	660	650	650	650
			120					135		

Table 9 (cont'd)

Properties of the oil	1; BHT, 0.01% of liver by weight	2; propylgallate, 0.01% of liver by weight	3 Control	4; BHT 0.015% of oil by weight	5; propylgallate, 0.001% of oil by weight	6 Control	7; BHT 0.015% of oil by weight	8 and 9; BHT 0.01% of the liver by weight	10; propylgallate 0.001% of liver by weight
Peroxide number, % iodine	0,11	0,14	0,1	0,087	0,089	0,135	0,115	0,1	0,13	0,165
Retention of oxiranic oxygen, mg. %	2,85	3,10	3,57	2,40	2,64	2,8	2,6	4	5	2,4
Carbonyl number	1,3	2,7	3,9	1,3	1,3	—	—	—	—	—
Thiobarbituric number	3,2	1,5	4	2,7	3,4	1,3	0,7	1,4	0,5	1,5
Acid number	0,7	2,2	2	1,2	1,5	1,7	1,9	2,3	1,4	1,4
Retention of Vitamin A, I.U. per gram	630	650	780	720	730	630	620	580	620	790
150 days										
Peroxide number, % iodine	0,08	0,18	0,17	0,1	0,17					
Retention of oxiranic oxygen, mg. %	3,8	5,5	3,3	2,8	5,5					
Thiobarbituric number	0,7	4,7	1,7	2,7	5,3					
Acid number	0,9	2,3	2,8	1,2	1,5					
Retention of Vitamin A, I.U. per gram	410	590	830	560	550					

* 0.015% BHT was retained in the separated oil
** 0.001% propylgallate was found in the separated oil

Table 10 /63

Organoleptic properties of the oil of Atlantic cod during storage

Test number	Antioxidant and its concentration	30 days	60 days	90 days	120 days	150 days
1	BHT*, 0.01% of liver, by weight	Odour of cod liver oil	Odour of cod liver oil	Odour of cod liver oil	Weak odour of oxidized oil	Weak odour of oxidized oil
2	Propylgallate** 0.001% of liver, by weight	No odour of oxidized oil	Odour of cod liver oil. Odour of melted butter	Weak odour of melted butter	Weak odour of melted butter	Odour of melted butter
3	Without antioxidant - control	No odour of oxidized oil	Weak odour of oxidized oil	Odour of oxidized oil with an odour of stale fish	Odour of oxidized oil with a strong odour of stale fish	Odour of oxidized oil with a strong odour of stale fish
4	BHT, 0.015% of oil, by weight	Same	Odour of cod liver oil	Odour of cod liver oil	Odour of cod liver oil	Weak odour of oxidized oil

* 0.015% BHT was retained in the separated oil.
** 0.001% propylgallate was found in the separated oil.

Table 10 (cont'd) /64

Test number	Antioxidant and its concentration	30 days	60 days	90 days	120 days	150 days
5	Propylgallate*** 0.001% of oil, by weight	No odour of oxidized oil	Odour of cod liver oil	Weak odour of oxidized oil	Weak odour of oxidized oil	Weak odour of oxidized oil
		45 days	75 days	105 days	-	135 days
6	Without antioxidant - control	Odour of stale fish	Slight odour of oxidized oil	Odour of oxidized oil with an odour of stale fish	-	Odour of oxidized oil and stale fish
7	BHT, 0.0015% of the oil, by weight	Odour of cod liver oil	Odour of slightly oxidized oil	Weak odour of oxidized oil	-	Weak odour of oxidized oil
8	Propylgallate, 0.001% of oil, by weight	Same	Same	Same	-	Same
9	BHT, 0.01%* of liver, by weight	Odour of cod liver oil	No odour of oxidized oil	Odour of cod liver oil		Odour of cod liver oil
10	Propylgallate** 0.001% of liver, by weight	Odour of stale fish	Odour of stale fish. Odour of melted butter	Odour of stale fish - no odour of oxidized oil		Odour of stale fish with a weak odour of oxidized oil

* 0.015% BHT was retained in the separated oil.
** 0.001% propylgallate was found in the separated oil.
*** The actual concentration of pure propylgallate is 0.0007%.

Table 11. /65

Changes in the oil of the residue upon storage under laboratory conditions

Test number	NaCl %	Antioxidant %	Composition of residue, %		Oil in the residue															
					Before storage				After 10 days of storage				After 20 days of storage				After 40 days of storage			
			oil	moisture content	peroxide number, % iodine	acid number	carbonyl number		peroxide number, % iodine	acid number	carbonyl number	organoleptic analysis	peroxide number, % iodine	acid number	carbonyl number	organoleptic analysis	peroxide number	acid number	carbonyl number	organoleptic analysis
4a	10	BHT, 0.01	31,5	53,6	0,06	0,8	—	0,06	0,8	—	Good odour and taste	0,12	1,5	—	At the top - odour of oxidized oil, good odour in centre	0;11	2,0	—	Mold, odour of oxidized oil
5a	10	BHT, 0.01	32,2	58,8	0,14	1,4	—	0,1	1,7	—	Same	0,09	1,7	—	Same	0,12	2,2	—	Same
1	5	-	3,35	52,8	0,09	1,1	—	0,18	1,6	5,5	Mold on the top, odour of oxidized oil	—	—	—	-	—	—	—	-
2	5	BHT, 0.01	32,6	54,4	0,05	1,1	—	0,03	0,9	2,4	Mold, weak odour of oxidized oil	—	—	—	-	—	—	—	-
3	8	BHT, 0.01	36,7	50,1	0,07	1,3		0,04	0,5	2,7	No signs of oxidation in odour or taste	0,09	0,8	3,4	No mold, normal odour	0,06	0,9	5,97	Mold on surface, weak odour of oxidized oil

Test number	NaCl %	Antioxidant %	Composition of residue, %		Oil in the residue													
					Before storage				After 10 days of storage				After 20 days of storage				After 40 days of storage			
			oil	moisture content	peroxide number, % iodine	acid number	carbonyl number		peroxide number, % iodine	acid number	carbonyl number	organoleptic analysis	peroxide number, % iodine	acid number	carbonyl number	organoleptic analysis	peroxide number	acid number	carbonyl number	organoleptic analysis
4	8	BHT, 0.01	32,4	58,9	0,1	0,9			0,08	0,7	3,7	Same	0,11	1,3	4,1	Same	0,16	1,2	8,7	Same
5	3	BHT, 0.01	32,6	57,3	0,09	1,8			Mold appeared on the third day of storage											
8	8	-	38,1	47,6	0,07	1,8			0,06	1,7	7,8	No mold, normal odour	0,1	1,9	6,9	Mold, odour of oxidized oil	-	-	-	-
9	8	BHA, 0.01	32,5	55,1	0,06	0,9			0,06	1,2	5,5	Same	0,08	1,4	5,8	Mold, no odour of oxidized oil	-	-	-	-
10	8	BHT, 0.01	—	—	0,023	1,9			0,026	1,1	4,5	Same	0,06	1,2	4,9	-	-	-	-	-