FROM STUDIES ON THE STABILITY AND NUTRITIVE VALUE OF EDIBLE FATS AND OILS

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1. Introduction

This part of the paper is designed to present a brief outline of the research activities of the Division of Food Science throughout the whole period of its existence. The first thing to be stressed is that the research projects done in the Division although diversified have mostly been intended for practical applications. As a matter of fact some studies involved basic research but practical side hase always been borne in mind.

One of the first research subjects were vitamins. The studies in this area involved verification and modification of analytical methods, the effect of storage conditions, preservation and processing on the stability of chosen vitamins and provitamins (L-ascorbic acid, riboflavin, carotenes) as well as characterization of some vitamin derivatives [1-8].

A number of studies were devoted to noxious substances occurring in foods and foodstuffs, such as pesticides, polycyclic aromatic compounds, nitrosoamines and aflatoxins. The studies carried out in this field concerned analytical methods, quantitative changes of these compounds and their degradation products in chosen foods (coffee beans, coffee substitutes, meat and meat products, milk and milk products, edible fats) and animal feeds during storage and processing [9-17].

As far as analytical methods are concerned special attention has been paid to organoleptic testing. For example using the multidimensional scaling the chief properties of meat and meat products were named, and the functional correlation between these properties was established, which made it possible to predict consumers' preferences for these products [18-21].

For about 15 years now the leading research area have been edible fats and oils. The studies carried out in this area included the problems of storage of oilseeds, oils and margarines, the effect of antioxidants and components of non-saponifiable fraction on the stability of fats and oils under different conditons of storage and thermal treatment. Attempts were also made to reduce the extent of polymerization and transisomerization of fatty acids in fat products subjected to severe heat treatment (frying, roasting). Further experiments in this area concerned the effect of different parameters of heat processing on the nature and extent of changes in the fat fraction

of different fat products. As reported below the applicability was proved of riboflavin preparations for enriching margarines [22-24, 27-30, 32-35, 41-43, 46].

A number of studies were devoted to packagings for fats and oils. In studies on the subject the protective properties were verified of the packaging materials used in this country, the best were named and certain modifications were proposed to increase the protective properties of some of them. Besides a set of indices was proposed for qualifying packaging materials for fats and oils [20, 23-26].

2. Changes of rapeseed oil fatty acids during heating and means for improving their thermostability

In spite of relatively low nutritive qualities high-erucic rapeseed oil (ERSO) still contributes to the human diet in this country. As a matter of fact refined and hydrogenated rapeseed oils are widely used both in commercial and household frying, roasting etc. It seems a common knowledge now that the low nutritive value of ERSO is due not only to the high content of erucic acid but also to naturally present goitrogenic compounds and unbalanced ratio of saturated to monounsaturated fatty acids. Quite a number of investigations were devoted to the effect of heat treatment on the nutritional qualities of rapeseed oils. Severe heating (frying, roasting) was proved to cause extensive changes in their sensory and nutritional qualities, and polymerized ERSO was reported to be much more harmful than polymerized soybean or sunflower oils.

In the present paper a review is given of the investigations carried out in this laboratory on the heat stability of ERSO. In most experiments the oils were heated isothermally within 110-200°C, and to insure correct concluding the kinetic presentation of results was applied. The rate and extent were followed in oxidation, degradation, polymerization and stereoisomerization of fatty acids. Besides the changes in the contents of individual fatty acids and, in some experiments, in the contents of characteristic protons were followed. The experiments were effected on samples of raw, refined and hydrogenated ERSO.

According to the results obtained the stage of processing influenced essentially the heat stability of ERSO. A relatively high stability was stated with the samples of raw and hydrogenated oils [28]. As far as the hydrogenated oil was concerned its stability was found to depend on the parameters of heating, first of all on the temperature, provided that the heating time was kept within the induction period. Beyond the induction period the changes were much more rapid, their rate resembling that in the refined oil. For example the samples of hydrogenated oil heated at 130°C exhibited a good oxidative stability up to the 7-th hour of heating; on further heating a pronounced increase in peroxides was observed. At 150°C a 2-hour heating was enough

to cause a significant increase in the oxidation rate [29]. Refined oil was found to be the most susceptible to oxidation, this being so not only during heating but also during storage [27, 28]. Similar differences were observed in the character and extent of polymerization. The changes observed in dynamic viscosity, mean molecular weight, refraction and in characteristic protons made it possible to conclude that heating led to the formation of interglyceride polymers in raw oil, of high amounts of polymers of fairly uniform molecular weight in refined oil, while in hydrogenated oil dominated branched polymers with interglyceride bonds.

The rate and extent of individual deteriorating processes were found to depend essentially on the heating temperature applied. In refined and hydrogenated oils heated in the range 110-130°C and in raw oil heated in the range 110-150°C hydrolytic cleavage and oxidation were predominant. Within the temperature range 150-200°C a drop in the rate of oxidation was observed, this being accompanied by a gradual decrease in peroxides. In the latter temperature range polymerization and transisomerization were predominant, and the higher was the heating temperature the higher was the rate of these processes [28, 29].

It should be stressed that of the two factors, heating time and temperature, the latter was by far more effective in promoting oxidation, polymerization and transiomerization [31].

A number of studies were aimed at improving the heat stability of chosen plant oils. To this end heating tests were carried out using samples of oils to which some compounds or materials were added to retard deterioration. For instance $SnCl_2 \cdot 2H_2O$ was used to reduce the rate and extent of polymerization in sunflower oil heated at 150°C. From among three concentrations tested, namely 50, 100 and 200 ppm, the later was found to have the best protective properties, this applying not only to polymerization but also to oxidation and trasisomerization [32]. On the other hand the addition of $SnCl_2 \cdot 2H_2O$ caused a slight increase in the rate of hydrolytic cleavage of triglycerides. In some other experiments certain chemically inactive agents were tried to inhibit polymerization.Because of the free radical character of polymerization some ceramic materials were used as agents deactivating free radical energy [33]. In a few series of experiments the following materials were tested: glass beads [34], porcelain plates and plates from porous glass [35]. The experiments were effected using the samples of refined and hydrogenated ERSO and of refined soybean oil, applying isothermal heating at 160°C, 180°C and 190°C. It appeared that the higher was the heating temperature the higher was the protective effect of the materials tested. The best results were obtained with the plates from porous glass. The latter plates were found to inhibit all the unfavorable changes in soybean oil heated at 180°C for 24 hours, decreasing the extent of:

- hydrolytic cleavage of triglycerides by 11%,
- oxidation by 37%,

- dynamic viscosity by 17%,
- refraction by 31%.

The most pronounced protective effect of porous glass concerned the degradation of fatty acids as determined by gas chromatography with an internal standard. The extent of degradation or polymerization of fatty acids dropped by 46%.It is the most reliable index because illustrates both the degree of polymerization and degradation of fatty acids. The plates from porous glass were found to inhibit transisomerization. In the samples of soybean oil heated for 24 hours at 180°C the increase in trans-isomers amounted to 2.9% while in those with a plate from porous glass only to 1.8%.

3. The effect of squalene on the heat stability of plant oils

The heat stability of oils and fats is believed to depend not only on their fatty acid pattern but also on the composition of the unsaponifiable fraction [36-39]. The addition of unsaponifiables isolated from wheat germ oil, corn oil, Vernonia anthelmintica or olive oil was found to retard oxidative polymerization in vegetable oils and model lipid systems subjected to heating [36, 37]. The protective effect of the unsaponifiable fraction is ascribed to sterols and methylsterols with an ethylidene group in the side chain [38, 39]. There is scant information on squalene as a potential oxidation inhibitor. According to Sims a. co. [36] a 0.5% addition of squalene markedly retarded the degradation of unsaturated fatty acids in sunflower oil heated at 180°C. As reported by Gowind a. co. [40] squalene initially showing antioxidant properties for methyl linoleate and methyl oleate heated at 63°C was found to promote their oxidation on prolonged heating. The experiments carried out in this laboratory proved squalene to inhibit oxidation in rapeseed and sunflower oils heated at 170°C (table 1) [41-43]. A detailed study was devoted to establishing the concentrations of squalene securing the highest heat stability of rapeseed oil. To this end changes were followed in samples of rapeseed oil containing squalene in concentrations from 0.10 to 2.25% and in those with no squalene added (controls) and heated at 170°C for 35 hrs. The analysis included the following indices:

- contents of individual fatty acids determined by gas chromatography,
- contents of polar compounds determined by IUPAC AOAC procedure [44],
- dynamic viscosity determined with a rotary viscosimeter Rheotest 2 at 40°C,
- peroxide value determined according to Lea.

The above set of indices made it possible to establish changes in the contents of individual fatty acids as well as in the rate and extent of oxidation and polymerization. Under the conditions applied the addition of squalene retarded the degradation of unsaturated fatty acids and limited the extent of polymerization, the highest protective effect being achieved with the squalene concentrations above 1%. One per

Table 1. Rate of deterioration of rapeseed and sunflower oils during heating at 170°C ($\bar{x}$)

Fatty acids and indices	Rapeseed oil			Sunflower oil		
	control	heated 24 h	+ 0.4% squalene heated 24 h	control	heated 24 h	+ 0.4% squalene heated 24 h
Oleic acid (mg/1 g fat)	592.2	523.2	525,8	409.8	307,9	316.1
Linoleic acid (mg/1 g fat)	259.6	180.5	193,9	431,2	299,3	308,5
Linolenic acid (mg/g fat)	105.2	53.5	65.1	1.0	traces	traces
Refractive index (n_D^{40})	1.4668	1.4720	1.4712	1.4663	1.4705	1.4705
Viscosity (η_{cP}^{40})	37.60	48.39	47.05	34.23	52.12	50.10
Polar compounds (%)	3.90	24.95	21,30	4.10	32.20	30.10

n = 4

cent addition was proposed as practically applicable; higher squalene concentrations gave no proportional increase in its inhibitory properties. With the time of heating the antioxidant activity of squalene decreased which suggested that oxidized squalene derivatives promoted oxidation. The oxidation of unsaturated faty acids and of unsaturated unsaponifiables is now believed to follow the same free radical course. Thus the oxidation products of unsaponifiable compounds and of fatty acids may act as catalysts for one another. To elucidate this catalytic interaction a research should be done on the changes that the unsaponifiables undergo during heat treatment and on the identity of the resulting products.

Some experiments were aimed at isolating and characterizing squalene oxidation products as well as at establishing their effect on the heat stability of rapeseed oil. An 8-hour heating of squalene at 170°C gave a mixture of products that could partially be separated by TLC and GC. Using IR and MS techniques the molecular weight of 3 products was established. The compounds isolated from the mixture included aliphatic ketones, isomers differing from one another in the number and position of double bonds as well as in the structure of carbon skeleton (table 2).

According to the results obtained a thermally oxidized squalene acts as a prooxidant. The addition of heated squalene, containing over 40% of degradation products,

Table 2. Characteristics of squalene oxidation products

No	Rf_{TLC}	RRT_{GLC}	H_2SO_4 (color)	IR (cm^{-1})	MS M^+
1	0.92	1.36	brown		
2	0.88	1.36	brown		
3a	0.75	0.56		3450, 1740, 1690, 1640	356
3b	0.75	1.34			426
4	0.66	1.54	brown	3450, 1660, 1640, 890	408
5	0.56	1.54	brown	3450, 890	

caused a drop in unsaturation and promoted polymerization in the samples of rapeseed oil heated at 170°C.

4. The applicability of riboflavin for enriching margarines

To replace butter in the diet pattern margarines must contain appropriate amounts of fat soluble vitamins. Since the processing of oils intended for margarines causes extensive losses of vitamins and provitamins, enriching these products has become a common practice. Most margarines produced for the consumer market are fortified with vitamins A and D, very often with betacarotene and sometimes with vitamin E [45]. The use of betacarotene serves a dual purpose of supplying a desirable color and vitamin A activity. The margarines produced in this country are fortified with vitamins A and D (table 3). With the object of improving the nutritive value of margarines a study was carried out in this laboratory on the applicability of riboflavin as an enriching ingredient for "Margaryna mleczna" and "Masło roślinne MR" [46]. To this end storage experiments were effected with the use of samples of these two products containing riboflavin in the amounts 2, 4 or 6 mg/100 g and of unenriched ones (controls). The analysis of the samples included the following indices:

- contents of riboflavin in fortified samples [47],
- development of microflora (total non-acidifying microorganisms, total molds and total yeasts [48],
- sensory qualities [49],
- changes in the fat fraction: hydrolytic cleavage of glycerides [50], oxidation [51] and polymerization [52].

Table 3. The contents of vitamins A and D in margarines according to the Polish Standard PN-77/A-86907

Vitamins	Margarine			
	"MR"	"Vita"	"Maja"	"Mleczna"
Contents of vitamin A (I.U./g)	30	20	30	20
Contents of vitamin D (I.U./g)	3	2	3	2

According to the results obtained riboflavin can be used as an enriching ingredient for "Margaryna mleczna" and "Masło roślinne MR" because:

a) the enriched samples were found to be stable sources of riboflavin. Although its contents gradually decreased with the time of storage the losses were relatively low and for the additions of 2, 4 and 6 mg/100 g amounted respectively to:

- 8.7%, 12.5% and 12.8% in the case of "Margaryna mleczna" on 40-day storage and
- 3.8%, 7.8% and 9.9% in the case of "Masło roślinne MR" on 35-day storage;

b) in the concentrations applied riboflavin slightly retarded the drop in organoleptic qualities of both products during storage;

c) the addition of riboflavin had no significant stimulative effect on the development of microflora and up to the end of the test (40 days) the degree of infection was much below the disqualifying values. In all the samples of both products the non-acidifying microorganisms were detected much earlier than molds or yeasts;

d) under the storage conditions applied the addition of riboflavin had no significant effect on the rate and extent of chemical changes in the fat fraction of both products:

- the values of acid number obtained proved the addition of riboflavin to have no significant effect on the rate and extent of hydrolytic cleavage of glycerides. In all the samples the contents of free fatty acids underwent minor variations with a slight increasing tendency. At the same time intervals the course of accumulation and secondary changes of free fatty acids in the enriched samples differed from that in the controls, the differentiation being too low however to conclude on the promoting or inhibitory effect of riboflavin,
- as measured by peroxide value the addition of riboflavin had a negligible effect on the extent of oxidation; the riboflavin enriched samples of "Masło roślinne MR" showed a somewhat higher resistance to oxidation than those of "Margaryna mleczna",
- under the storage conditions applied the samples of both products underwent insignificant polymerization and the addition of riboflavin had no effect whatsoever on the rate and extent of this process.

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Z BADAŃ NAD STABILNOŚCIĄ I WARTOŚCIĄ ODŻYWCZĄ ROŚLINNYCH TŁUSZCZÓW JADALNYCH

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Streszczenie

Przedstawione wyniki badań nad termostabilnością i wartością odżywczą tłuszczów roślinnych uzyskano w ramach trzech odrębnych tematów.

1. Zmiany kwasów tłuszczowych (KT) oleju rzepakowego w czasie ogrzewania i sposoby podwyższenia ich termostabilności. Ustalono charakter i zasięg zmian KT oleju rzepakowego w czasie ogrzewania w różnych warunkach oraz próbowano określić charakter powstałych polimerów. Wykazano też hamujący wpływ $SnCl_2$, perełek szklanych i porowatego szkła na przebieg polimeryzacji w ogrzewanych olejach roślinnych.
2. Wpływ skwalenu na stabilność termiczną olejów roślinnych. W oparciu o dane literaturowe i wyniki własnych badań określono dawkę skwalenu wyraźnie ograniczającą degradację KT i zmiany polimeryzacyjne w ogrzewanym oleju rzepakowym i słonecznikowym. Podjęto próbę identyfikacji produktów odbudowy skwalenu i udowodniono prooksydatywny charakter utlenionego termicznie skwalenu.
3. Przydatność ryboflawiny do fortyfikacji margaryn. Na podstawie zmian jakości prób masła roślinnego MR i margaryny mlecznej z dodatkiem i bez dodatku ryboflawiny w warunkach testu przechowalniczego udowodniono przydatność preparatów tej witaminy do fortyfikacji obu rodzajów margaryn i określono właściwe dawki.

К ВОПРОСУ О СТАБИЛЬНОСТИ И ПИТАТЕЛЬНОЙ ЦЕННОСТИ ПИЩЕВЫХ ЖИРОВ РАСТИТЕЛЬНОГО ПРОИСХОЖДЕНИЯ

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Резюме

Представлены результаты исследований термостабильности и питательной ценности пищевых жиров растительного происхождения, полученные в рамках трёх отдельных проблем.

1. Изменение жирных кислот (ЖК) рапсового масла во время их нагревания и способы повышения их термостабильности. Установлен характер и масштабы изменений ЖК рапсового масла во время нагревания в разных условиях, а также определялся характер возникших полимеров. Доказано тормозящее влияние $SnCl_2$, стеклянных шариков и пористого стекла на ход полтмеризации в нагреваемом растительном масле.

2. Влияние сквалена на термостабильность растительных масел. Опираясь на данные литературы и результаты собственных исследований была определена доза сквалена, отчётливо ограничивающая распад ЖК и полимеризационные изменения в нагреваемом рапсовом и подсолнечном масле. Бяли предприняты попыеки отождествления продуктов распада сквалена и был доказан проокислительный характер термически окисленного сквалена.

3. Пригодность рыбофлавина для фортификации маргаринов. На основе изменений качества исследуемых маргаринов с добавлением и без добавления рыбофлавина при хранении была доказана пригодность препаратов этого витамина для фортификации исследуемых мвргаринов и были определны соответствующие дозы.