

Study of lipide structures from the aspects of their nutritive value and technological quality*

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5. Summary

- 5.0 The theses of my doctor's dissertation entitled "Study of lipide structures from the aspects of their nutritive value and technological quality" evolve from the results of works from the period 1955—1981. They are based on results of three Faculty, six State (partial) and two conclusive State Research Projects, inclusive more than 100 publications, 5 patents and 4 improvement technological processes for the industry of fats. Several of the findings were incorporated into two scripts.
- 5.0.1 These theses concern a more complex investigation of relationships of geometric *cis*- and *trans*-isomers (TI) of unsaturated fatty acids, especially *trans*-9-octadecenoic (elaidic), from the aspect of their biological equality, their formation and possibilities of simplified control in industrial, partial catalytic hydrogenation (PCH) and also their transfer to the process of intermolecular pre-esterification of triacylglycerols, which can be a potential PCH substituent in industrial production of edible fats without TI.
- 5.1 For this purpose a complete review of literature elaborated was oriented to:
- 5.1.1 biological properties of unsaturated fatty acids and their geometric TI,
- 5.1.2 questions concerning partial catalytic hydrogenation and its catalysts (homogeneous and heterogeneous), relationships with TI formation, selectivity, the method of TI determination and their selectivities,

*Summary of the doctor's dissertation thesis.

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- 5.1.3 significance, possibilities and methods of intermolecular esterification (animal and vegetable fats) and more exact methods for TAG structure determination, reaction dependences and properties of the final product of esterification reaction.
- 5.2 Experimental results can be summarized as follows:
 - 5.2.1 The biological experiment using the microbiologic model *Candida albicans* showed again the degradability of individual fatty acids as sources of carbon, and that according to the rate of growth of curves these acids have the following order: oleic acid, glucose, stearic and elaidic acids.
 - 5.2.2 *trans*-3-Dodecenoic acid, which is the assumed non-degradable remnant of the starting *trans*-9-octadecenoic (elaidic) acid, was synthesized using the Witting reaction and then used in the nutrient medium *C. albicans*. It was shown intact and did not manifest any changes in the height of the curve growth.
 - 5.2.3 Microorganism *C. albicans* seems to be a suitable model for distinguishing *cis*- and *trans*-isomers of oleic acid.
 - 5.2.4 As far as definite conclusions are concerned it is the verification of the influence of synthesized *trans*-3-dodecenoic acid that seems to be necessary for the function and histology of the liver macroorganism.
- 5.3 Investigation of conditions and possibilities of evaluating the process of partial catalytic hydrogenation showed that:
 - 5.3.1 The experimentally determined TI contents in the case of laboratory PCH reached about 50%, in industrial PCH it was as much as 70%.
 - 5.3.2 By lowering the ratio between the re-used and the fresh catalyst (by lowering the number of repeatedly used Ni catalyst — three times maximally) it was possible to decrease TI content only through minimal changes, or through changes in the technological reaction of PCH.
 - 5.3.3 The possibility of an extended technique of expressing PCH selectivity was verified. Consequently, it was found advantageous to use the parameter of selectivity expressed qualitatively and quantitatively by a new value, which means the whole or complex selectivity (CVS). For CVS classified values of rate constants: trienes (T), dienes (D), monoenes (M) as well as *cis*- and *trans*-isomers have to be used as follows:
 - 5.3.3.1 numerically,
 - 5.3.3.2 graphically.
 - 5.3.3.1.1 The computer programme CATSEL elaborated in advance, was completed by a subprogramme for numerical determination of quantitative relations of CVS percentual values.
 - 5.3.3.2.1 CVS can be graphically interpreted using polygonal diagrams inscribed

in circles with the unit radius (5 cm) from the smallest triangular to the greatest sixteen-angular diagram.

- 5.3.4 The correlation relationships between the numerical and graphical methods of percentual determination of CVS were solved and found acceptable.
- 5.3.5 From the great collection of samples (rape seed oil, soya bean and sunflower oils, hydrogenated under different conditions in laboratory and industry conditions with our commercial Ni catalyst and imported Nysel DM-3 catalyst, after a computation using the CATSEL programme — that is, computation of rate constants D, T, M and *cis*- and *trans*-isomers, the greatest probability of their range was determined.
- 5.3.5.1 Accordingly, the rate constants have most frequently the values: T = 0 to 4, D = 0 to 2, M = 0 to 0.15, *trans*-isomer = 0 to 2, and *cis*-isomer = 0 to 1 (h^{-1}).
- 5.3.5.2 In extreme pressure, temperature and catalyst concentration conditions, the rate constants must be determined separately.
- 5.4 The ways of a systematic determination of TI content:
- 5.4.1 Using the computer programme TRANSC empirical equations with two and three parameters were obtained, with whose help TI content in industrially hydrogenated rape seed oil and in sunflower oil was determined by computation only after substitution of: PVC temperature, time, eventually a unique experimental value — the melting point of a product. The solution of this empirical equation using a pocket calculator took 5 to 10 min.
- 5.4.2 By solving empirical equations with the A72T2B tabular programme, which was also elaborated, we obtain tables from which TI content in industrially hydrogenated rape seed and sunflower oils (products of n. e. Palma) can be determined by a simple systematical subtraction of the known PCV time, PCV reaction temperature and melting point of a product. This can be done by an unqualified worker directly in the working process, at any time of PCV reaction.
- 5.4.2.1 Tables for subtracting TI can be prepared for every kind of hydrogenated oil and for any hydrogenating reactor, respectively. To this end some experimentally measured values of TI in IR spectrum region may serve as starting basis.
- 5.5 To determine the parameter selectivity of PCH process a new computing programme named CRUTIL has been elaborated that on the basis of calculated rate constants makes it possible to compute: concentration changes of T, D, M, S; in the case of rape seed oil also concentration changes of erucic, eicosenic, behenic acids, and *cis*- and *trans*-isomers; all differences of final ratios to initial concentrations; the

- corresponding physico-chemical values: IV, ThV and RI (refraction index) and their differences; values of hydrogenation rate, isomerization rate, isomerization indices and isomer ratios; and finally function dependences of obtained physico-chemical values from concentration changes of individual fatty acids as well as their isomers with the form of the resulting polynomial equation of second degree with the corresponding coefficient of correlation R.
- 5.6 A large number of computation results enabled construction of the following diagrams:
 - 5.6.1 Diagram of dependence IV and the fraction of D/D₀ concentration that makes it possible to determine the D₂₁ selectivity of the first degree in hydrogenated sunflower oil;
 - 5.6.2 Diagram of dependence IV = f(M/M₀), for subtracting the monoene/saturate selectivity ratio in hydrogenated sunflower oil;
 - 5.6.3 Diagram of dependence D = f(log M), for subtracting S₂₁ selectivity ratio in hydrogenated sunflower oil only by means of final concentrations of D and M (after one GLC analysis only);
 - 5.6.4 Diagram of dependence M = f(log M₀), for subtracting S_{MM0} selectivity ratio in hydrogenated sunflower oil in the same way but only by means of final concentrations of monoenes and saturates;
 - 5.6.5 Diagram of dependence T = f(log D), for subtracting S₃₂ selectivity ratio in hydrogenated rape seed oil with a high content of erucic acid;
 - 5.6.6 Diagram of dependence T = f(log D), for subtracting S₃₂ selectivity ratio in hydrogenated rape seed oil with a low content of erucic acid but only by means of final concentrations of trienes and dienes.
 - 5.7 On the basis of hitherto known findings and results obtained in this field it is to be assumed that in the next future we shall dispose of
 - 5.7.1 A system of diagrams that would make it possible to subtract rate constants of all fatty acids in the reaction scheme of both dienes and trienes, as well as *cis*- and *trans*-isomers.
 - 5.7.2 The determination of complete values' selectivities (CVS) using combination of diagrams from the point 5.6 and diagrams of polygonal conception (5.3.3.2.1) will be possible only on the basis of simple determinations of percentual values (CVS) with the help of a system of easily read diagrams.
 - 5.7.3 Preparation of tables would make it possible to determine more exactly all rate constants in tirene and diene types of triacylglycerols by simple subtraction.
 - 5.7.3.1 By qualifying the individual rate constants we obtain the percentual value of complex selectivity directly or by making a combined use of a polygonal conception — graphically permanently.

- 5.7.4 The most important and at the same time most complex parameters of PCH, i.e. selectivity, activity of catalysts and TI formation are expected to be observed systematically, rapidly and exactly even by an unqualified worker without using an otherwise unavoidable, necessary computation technique.
- 5.8 The methods used in this work and the results obtained were stimuli for the purpose to elaborate a procedure in which all the decisive data related to PCH would be available only on the basis of experimentally, most simply determinable physical value — namely that of the index of refraction.
- 5.9 The triacylglycerol selectivity has not been studied. Its exact determination will, however, be possible on the basis of changes determined in trisaturated structural forms of TAG molecules or TAG with a definite number of double bonds in TAG molecules. Such interpretations are also available in using the already elaborated computation programme TRIGST, when qualitative and quantitative values of fatty acids, positionally determined by stereospecifically reacting pancreatic lipase, are used as input data.
- 5.9.1 Consequently, we have at our disposal a methodologic procedure which makes it possible for us to observe selectivities of hydrogenated fats in their complete scale, completely, simply and exactly.
- 5.10 In the field of intermolecular pre-esterification (IMPE) an uncontrolled type of reaction of animal fats (tallow and lard) has been studied. These fats themselves are not optimal from the aspect of rational nourishment.
- 5.10.1 Animal fats were used in different ratios to vegetable oils (peanut and sunflower oils) using different catalysts: CH_3ONa , Zn, Mg, $\text{NaOH} + \text{glycerol}$, Sn, BF_3 in relation to the resulting melting point of pre-esterified products.
- 5.11 From the catalysts compared it was CH_3ONa that had the greatest influence on the decrease of the melting point of the pre-esterified product; BF_3 had the greatest influence on the increase of the melting point value. $\text{NaOH} + \text{glycerol}$ and Zn caused temporary increase, and after some time, tendency of lasting decrease. Mg decreased the melting point value almost linearly and Sn brought about decrease quite linearly with time.
- 5.11.1 The highest value of the melting point in the mixture tallow : sunflower oil = 1 : 2, at 32°C , was obtained with BF_3 catalyst as soon as in 30 min, the lowest value of the melting point of the same mixture with CH_3ONa catalyst, at 20°C , as late as in 3 h.
- 5.12 Optimal ratios between the contents of essential fatty acids and con-

- sistence of a pre-esterified product were obtained with ratios 35 to 40% of tallow, and 60 to 65% of sunflower oil.
- 5.13 As far as TI content was concerned, their presence in all samples of experimentally tested pre-esterification was found negative.
 - 5.14 All pre-esterified samples were subjected to a complex structural analysis of triacylglycerols.
 - 5.14.1 After a stereoscopic hydrolysis with pancreatic lipase, TLC separation and an isolation of fatty acids from C-2 and C-1,3-positions, their qualitative and quantitative ratio was determined by GLC analysis.
 - 5.14.2 All samples prepared by pre-esterification were studied by a complex structural analysis by means of the computation programme TRIGST.
 - 5.15 We obtained numerical interpretations of TAG structures of the following types: mixed AAA to ABC, aligned SSS to UUU, and structures with definite numbers of double bonds in TAG molecules (N) from $N = 0$ to $N = 9$.
 - 5.15.1 We also obtained quantitative relationships of these structures as well as their numerical and graphical interpretations concerning the representation of individual fatty acids in determined TAG positions (so-called individual ATG structures).
 - 5.16 Possibilities of mathematical regression analysis, more exactly functional dependences of individual TAG structures on physico-chemical values and representation of the essential fatty acids, were found.
 - 5.16.1 Dependences with one variable (of the type: melting point = $f(\% \text{ SSS})$) provided satisfactory coefficients of correlations only on the level of approximations with polynomial of the fourth degree.
 - 5.16.2 Dependence of individual types of TAG structures of pre-esterified products, approximated by polynomial of the second degree with parameters: IV, melting point and fraction of unsaturated and saturated fatty acids, provided triparameter empirical equations with correlation coefficients ranging from 0.80 to 0.98.
 - 5.16.3 Dependences of TAG structures on physico-chemical values (as well as essential fatty acids' content) of products of pre-esterification of animal fats with vegetable oils converged to possible stock composition of beforehand determined physical, chemical and biological properties of final products of the pre-esterification process.
 - 5.17 To separate and isolate products of TAG hydrolysis with pancreatic lipase, instead of the hitherto used TLC a new, more exact and reproducible technique has been worked out, based on the exploitation of high pressure liquid chromatography.
 - 5.17.1 The necessary equipment was adapted for this purpose. Tests, using model mixtures were favourable.

- 5.18 On a world-wide scale, there are more than 1 million tons of edible fats produced each year, using the process of pre-esterification. Still, there are many reasons why this amount should be increased in the future.
- 5.18.1 Proceedings used in this work and results and findings obtained on their basis would make it possible to prepare aids (diagrams and tables), with the help of which it will be possible to determine reaction conditions of the process of pre-esterification and the sum of resulting properties of the product quickly, simply and without the necessity of using computation technique.
- 5.19 Results and findings from the aspect of their possible exploitation:
- 5.19.1 As far as demands for rational nutrition and its preventive measures are concerned, it would be necessary — similarly as is the case with erucic acid — to control systematically TI content in hydrogenated fats and to declare it in edible fats produced from them. Methods designed and described in this thesis would make this practicable.
- 5.19.2 Immediate and reproducible decrease of TI level in industrially hydrogenated vegetable oils with a heterogeneous Ni catalyst showed to be implementable already with respect to the decrease in the number of cycles of repeated use of the catalyst, or in the ratio of the cycling catalyst to the fresh one.
- 5.19.3 From the aspect of nutritive values, technological complexity, economy and possibility to modify the physico-chemical properties of products, it is possible in industrial production of edible fats to substitute the PCH process by the process of intermolecular pre-esterification of vegetable oils with animal fats.
- 5.19.3.1 The entirely eliminated TI content (provided the original levels of fatty acids remain unchanged) and the optimal physico-chemical properties of final products are preconditions for a larger industrial production of solid edible fats on the basis of nutritionally, technologically and economically more favourable process of intermolecular pre-esterification of vegetable oils with animal fats.
- 5.19.4 Increased applicability of proceedings of the study of function dependence of properties and structures of TAG is easy to achieve and it can significantly contribute both to basic and applied research as well as to industrial production of edible fats.
- 5.19.5 From the aspect of eliminated TI content in industrially produced edible fats, the combination in which first total hydrogenation and then intermolecular pre-esterification with original vegetable oil are applied, is realizable.
- 5.20 Supplements to the thesis are:
- 5.20.1 A collection of scientific and scientific-popular papers published up

to the present, with summaries of reports presented at conferences and seminars, and conclusions of partial and final reports of successfully implemented Faculty and State Research Projects.

- 5.20.2 Complete tables for the subtraction of TI values in industrially hydrogenated sunflower and rape seed oils (products of n.e. Palma), provided the following values are known: reaction temperature, hydrogenation time, and melting point of the product (elaborated after completion of the manuscript to the time of its presentation).
- 5.20.3 In the whole context of the work there are six larger computation programmes elaborated — applicable to the study of technological PCH dependences (changes of basic values, activity and selectivity of catalysts), eventually to the study of TAG structure changes, and fourteen smaller computation programmes — oriented to the solution of some linear and non-linear dependences of changes of physico-chemical values in fats and oils. Complete listing of these computation programmes are available by request.

Структуры липидов в связи с их пищевой ценностью и технологическим качеством

Резюме

С учетом возможности более быстрой передачи информации приводится резюме результатов и данных докторской диссертации на тему: „Структуры липидов в связи с их пищевой ценностью и технологическим качеством“. Результаты охватывают следующий из комплекса взглядов: доказательство биологической дифференциации вторичных трансизомеров жирных кислот, их упрощенной проверки новыми методами во время процесса парциальной каталитической гидрогенизации и их минимизации в ходе нее вплоть до полной элиминации путем предлагаемого аналога пластических пищевых жиров межмолекулярной переэтерификацией, включая возможности управления ею с целью получения требуемых финальных свойств продукта.

Štruktúry lipidov vo vzťahu k ich výživovej hodnote a technologickej kvalite

Súhrn

Vzhľadom na možnosť rýchlejšieho prenosu informácií uvádza sa súhrn výsledkov a poznatkov doktorskej dizertácie vypracovanej na tému „Štruktúry lipidov vo vzťahu k ich výživovej hodnote a technologickej kvalite“. Výsledky z komplexu pohľadov zahrnujú: dôkaz biologickej diferenciacie sekundárnych *trans*-izomérov mastných kyselín, ich zjednodušenú kontrolu novými spôsobmi počas procesu parciálnej katalytickej hydrogencie a ich minimalizáciu počas nej, až ich úplnú elimináciu navrhovaným analógom výroby plastických jedlých tukov medzimolekulovou preesterifikáciou včítane možnosti jej riadenia na požadované výsledné vlastnosti produktu.