UNIVERSITY POLITEHNICA OF BUCHAREST
Applied Chemistry and Materials Science Doctoral School
Chemical and Biochemical Engineering Department

SEPARATION OF OMEGA FATTY ACID ESTERS BY MOLECULAR DISTILLATION

Abstract of the thesis

PhD Student:
Ing. Cristina Emanuela ADAM (ENĂŞCUŢĂ)

Supervisor:
Prof. dr. ing. Grigore BOZGA

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KEYWORDS: esters, polyunsaturated fatty acid, fish wastes , fish oils, omega-3 fatty acids, esterification, transesterification, acid catalyst, base catalyst, vapor pressure, vacuum distillation, molecular distillation, mathematical modelling

Note:
The notations of chapters, subchapters, figures and tables referenced in this document are the same as those in the Ph thesis.

INTRODUCTION

In agreement with the major interests manifested in recent years, the identification of new animal or vegetable sources to obtain the compound used in the fields of pharmaceutical, food and industrial and exploit them fully, this paper aims is to develop new processes for the bioresources valorization in order to obtain the omega-3 polyunsaturated fatty acid esters.

Fatty acids are important nutrients for the body's life, and the polyunsaturated fatty acids (PUFAs) are essential for human metabolism, especially those of omega-3 type such as eicosapentaenoic (EPA, C20:5) and docosahexaenoic acids (DHA, C22:6). These fatty acids present benefical effects against diseases such as coronary artery disease, inflammation, hypertension.

The omega-3 fatty acid esters are thermolabile substances with very low volatility and relatively close boiling points. One of the widespread techniques of separating this compound group from complex mixtures from which it is obtained is advanced vacuum distillation and in particular molecular distillation.

The documentation study about the status of the research related to the production and purification of the omega-3 fatty acid esters led to the following objectives:

- Analysis and improvement methods for obtaining oils with high content of the omega-3 polyunsaturated fatty acids, especially from the residues resulting from fish processing;
- Elaboration of specific procedures for the synthesis of the omega-3 polyunsaturated fatty acids esters from such oils;
- Development of techniques for enrichment mixtures of the omega-3 polyunsaturated fatty acids esters by vacuum distillation and molecular distillation;
- Development of mathematical models for separation of the esters mixtures by molecular distillation.

This thesis is structured into four chapters, an introduction section, a general conclusions section and a paper references section (the list of published and consulted papers).

Chapter 1 of the thesis presents a synthesis of the main publications from the literature regarding the subject of the thesis: sources of the fatty acids with high content of omega-3, processes for the separation of the oils rich in glycerides of these acids, physico-chemical processing of oils in order to obtain the fatty acids alkyl esters and finally, methods to enrich these esters mixtures in omega-3, with emphasis on advanced vacuum distillation methods, particularly molecular distillation.

In Chapter 2, it is widely described the obtaining and characterization of the polyunsaturated fatty acids ethyl esters derived from fish and microalgae oils, oils with high content of the omega-3 acids glycerides.

They were done consecutively: (i) the extraction of oils rich in omega-3 compounds, from microalgae and fish wastes; (ii) oils purification at the level required for their further processing; (iii) a preliminary step of the esterification with ethanol in acid catalysis of the free fatty acids present in important concentrations in these oils, acids which disturbs the synthesis by transesterification of the fatty acids alkyl esters; (iv) oils transesterification with ethanol in base catalysis; (v) polyunsaturated fatty acids esters purification previousely obtained (ethanol washing, discoloration and drying); (vi) concentration of this polyunsaturated fatty acids in omega-3 fatty acid esters by vacuum distillation and molecular distillation. This step is detailed in Chapter 4 of the thesis, together with the mathematical modeling studies of molecular distillation; (vii) elaboration and experimental testing of a process for obtaining an oil with a high content of polyunsaturated fatty acids by the omega-3 fatty acids ethyl esters transesterification with glycerine.

The equipment used in this stage are in the "Bioresurse" laboratory from ICECHIM and the "Innovative Products and Processes" laboratory from the Department of Chemical and Biochemical Engineering (UPB) respectively.

In the same chapter are presented details of the compositional analysis performed in the studies described above.

In **Chapter 3** are presented the results of the study of fatty acid esters physical properties determination: existing literature data, published methods for the estimating these properties, and comparisons between their predictions using chemical engineering software (COSMOtherm, AspenPlus and Simulis respectively).

Since the main parameters involved in the distillation separation process are boiling temperature and vapor pressure, a greater emphasis has been placed on determining these properties. Evaluation of the boiling temperature prediction for blends of the fatty acids alkyl esters and by-products occurring in the transesterification process (methanol, glycerine) were done using the COSMOtherm, Aspen Plus and Simulis programs. The predictions of the last two were slightly superior to those obtained with COSMOtherm.

Following the study, for the vapor pressures calculation, the Ceriani-Meirelles method was selected to study the modeling and simulation of molecular distillation. In order to evaluate the accuracy of this method, a comparison was made between its predictions and the experimental values obtained in this work, on a modern vapor-liquid equilibrium equipment VLE 50 bar. The results confirmed the good agreement for the estimating of the fatty acid esters vapor pressures.

Chapter 4 describes the results of experimental and theoretical studies of the process of concentration the ethyl esters mixtures in omega-3 esters.

The adopted separation procedure consists in two stages, the first is vacuum distillation (2.5-5.8 Pa) and molecular distillation the second (0.1-0.25 Pa).

To achieve the two stages of distillation, the specially designed equipment, existing in the department of "Chemical and Biochemical Engineering", the laboratory "Innovative Processes and Products" was used. In this chapter are presented in detail, description of equipment usage, working mode, operating parameters and separation performance. The obtained concentrate has a rich content of omega-3 fatty acids up to 87% (mass).

In the last part of this chapter are described the mathematical modeling and simulation of the molecular distillation operation.

A first modeling study is based on an analytical approach using mass balance equations combined with prediction of the evaporation rate given by Langmuir-Knudsen relations and vapor pressures with the Ceriani-Meirelles relationship.

The simulations result in different working conditions, reveal a concordance of the light and heavy fractions flows, respectively of the compositions in these fractions, with the experimental data within the error limits characterizing the experimental data.

A second modelling study is based on the use of the Artificial Neural Network (RNA) method. There are two types of networks built with one and two layers neurons, which describes the dependence of the total concentration of omega-3 esters on bottom fraction resulting from the molecular distillation on the evaporation temperature and the feed rate respectively. The model thus developed is used to optimize the molecular distillation process.

1. THE ACTUAL RESERCH STATUS REGARDING THE OBTAINING OF OMEGA ESTERS TYPE FROM BIORESURCE

1.1 Overview

Animal or vegetable fats are mixtures of triglycerides of various fatty acids (FAs). The types of fatty acids in their composition make them liquid or solid. At the room temperature, liquid triglycerides contain predominantly unsaturated fatty acids, while solid triglycerides contain predominantly saturated fatty acids. Oils and fats may have a terrestrial or aquatic origin, may be edible or inedible, the inedible being used in cosmetics, paint and varnish etc. Edible oils, such as peanut oil, mustard oil, sunflower oil, etc. are generally used in the food industry. In addition, some edible oils are also used to produce unsaturated fatty acids (Yang et al., 2004; Goli et al., 2008a; Wu et al., 2008).

Fatty acids are carboxylic acids with straight-chain, saturated or unsaturated (having one or more double bonds) and its length ranging between 4 and 36 carbon atoms. The structure of the fatty acids contains at the end of the chain a -CH_3 group, starting from the double bonds (known as the termination ω), and at the other end is an (carboxyl) -COOH acid group. The most common fatty acids are those with chain ranging from 12 to 24 carbon atoms. Of these, fatty

acids with 16 or 18 carbon atoms (palmitic and stearic) are most common in plants and animals. Unsaturated fatty acids represent more than half of the total lipid of plants and animals.

Glycerin esters with fatty acids (triglycerides) are the usual sources of fatty acids or esters of these with other alcohols. By transesterification of triglycerides with lower alcohols (C1-C2) fatty acids methyl or ethyl esters are obtained (R-$COOCH_3$ or R-$COOC_2H_5$). Fatty acid methyl esters obtained by transesterification of triglycerides from fat with methanol enter in the biodiesel fuel composition.

Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs) that contain double bonds (C=C) starting with the third carbon atom and continuing towards the end of the chain. They are found most frequently in plants, animals, algae, fungi or bacteria. Commercialy, are obtained from seeds of different plants or from marine sources.

The structural formulas of the most commonly known omega-3 fatty acids ethyl esters of are described in figures 1.1-1.6.

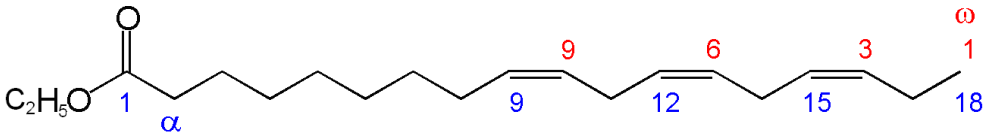

Fig. 1.1 cis-9,12,15-octadecatrienoic acid etil ester, C18:3 (ALA)

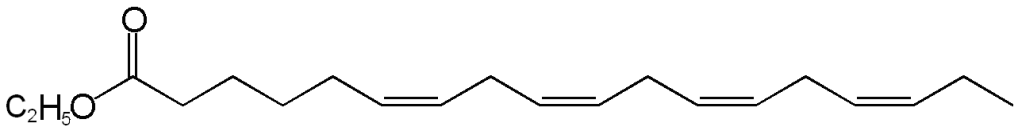

Fig. 1.2 cis-6,9,12,15-octadecatetraenoic acid etil ester, C18:4 (SDA)

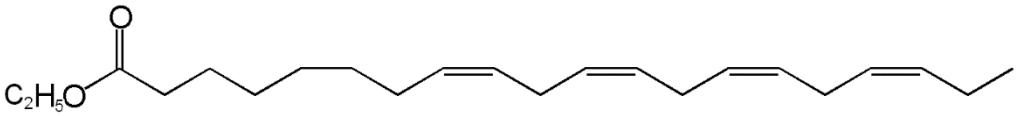

Fig. 1.3 cis-8,11,14,17-eicosatetraenoic acid etil ester, C20:4 (ETA)

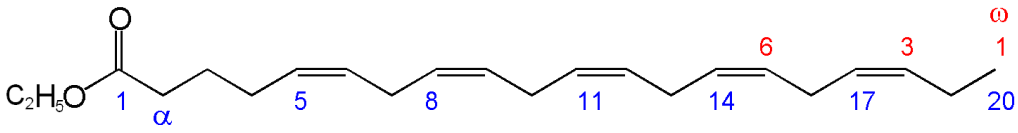

Fig. 1.4 cis-5,8,11,14,17-eicosapentaenoic acid etil ester, C20:5 (EPA)

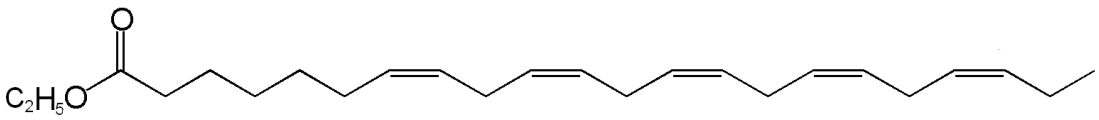

Fig. 1.5 cis-1.5 7,10,13,16,19-docosapentaenoic acid etil ester, C22:5 (DPA)

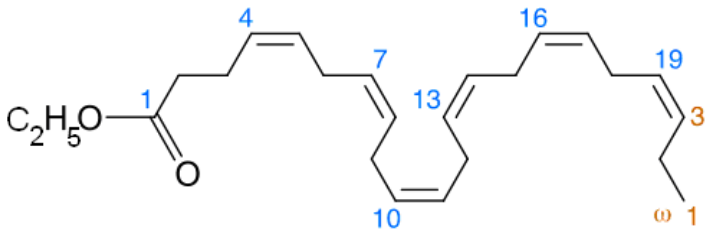

Fig. 1.6 cis-1.6 4,7,10,13,16,19-docosahexaenoic acid etil ester, C22:6 (DHA)

Thesis objectives:

- Analysis and improvement methods to obtain oils with high content of the omega 3 polyunsaturated fatty acids, especially from the residues resulting from fish processing;
- Elaboration of specific procedures for the synthesis of the omega-3 polyunsaturated fatty acids esters from such oils;
- Development of techniques for enrichment mixtures of the omega-3 polyunsaturated fatty acids esters by vacuum distillation and molecular distillation;
- Development of mathematical models of the esters mixtures separation by molecular distillation.

2. OBTAINING AND CHARACTERIZATION OF THE POLYUNSATURATED FATTY ACID ESTERS

2.4 Esterification of the free fatty acid from the oils rich in polyunsaturated fatty acids

Acid value of extracted fish oil from waste is 16.94±0.5 mg KOH/g. In order to improve the oil transesterification process (avoiding soap formation) and an increase the FAEE final yield, it was necessary a preliminary step of the free fatty acids (FFA) esterification.

The esterification of the free fatty acids with ethanol over solid superacid catalysts SO_4^{2-}/SnO_2-ZrO_2 was performed at atmospheric pressure, in a similar reaction setup already presented by *Stepan E., Enascuta C., et al., (2016).* In order to study the influence of reaction parameters on FFA esterification, the methodology of the D-optimal program was used (Adekoya et al., 2015). The dependence of the acid value on the ethanol:FFA molar ratio and catalyst concentration was studied.

The general second grade statistical model, is described by equation (2.2)

$$y = a + b_1 \cdot x_1 + b_2 \cdot x_2 + c \cdot x_1 \cdot x_2 + d_1 \cdot x_1^2 + d_2 \cdot x_2^2 \qquad (2.2)$$

$$x_i = \frac{v_i - v_{imin}}{v_{imax} - v_{imin}}$$

where:

y – prediction of the model (acid value); v_1 - molar etanol: FFA ratio (1 - 12) ; v_2 - catalyst amount (1-10 % mass).

The equation used to calculate the conversion of the free fatty acid was:

$$C_{FFA} = (A_{initial\ FFA} - A_{final\ FFA}) / A_{initial\ FFA} \times 100 \qquad (2.3)$$

where, $A_{initial\ FFA}$ is the initial acid value (mg KOH/g) and $A_{final\ FFA}$ is the final acid value (mg KOH/g).

The experimental design, using the Box-Behnken methodology is presented in Table 2.4. The seven experiments were performed according to the experimental program, and the experiments no. 2, 4 and 7 were replicated for a verification of the correctness of the measurements.

The results of the esterification of the FFA from fish oil with ethanol over SO42-/SnO2-ZrO2(3.0 Zr) are presented in Fig. 2.7.

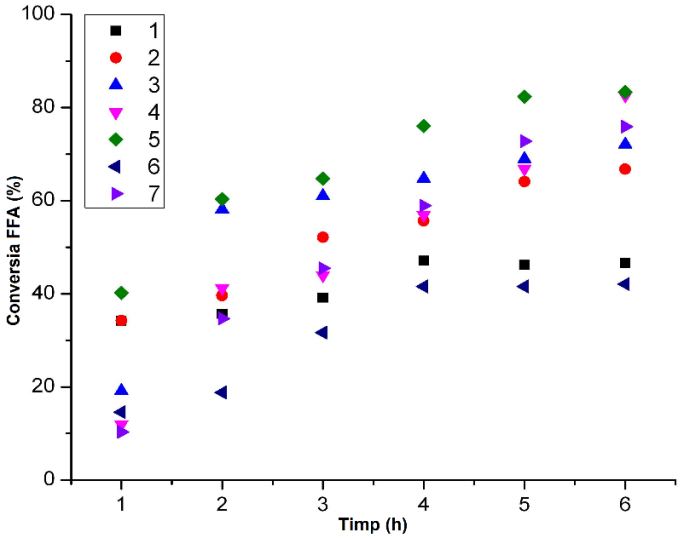

Fig. 2.7 The influence of reaction parameters over conversion of FFA (Enascuta et al. 2017)

As shown, the conversion of FFA is positively influenced by the catalyst mass, increasing almost linearly with the increase of catalyst amount. In the absence of catalyst, the conversion of FFA is very low. The highest FFA conversion was recorded for 10 wt.% of SO_4^{2-}/SnO_2-ZrO_2 catalyst at molar ratio ethanol to oil of 12.

3. DETERMINATION OF PHYSICAL PROPERTIES OF THE FATTY ACID ESTERS

3.1 Determination of the fatty acid esters boiling temperature

3.1.1 The experimental evaluation of the vapor pressure calculation method from group contributions, proposed by Ceriani si Meirelles (2004)

Besides the classical correlation of the vapor pressure temperatures, Antoine equation type, have been proposed additional specific methods for the class of fatty acid esters. Of these, is worth mentioning the method proposed by Ceriani and Meirelles (2004), based on group contributions.

In the following, an experimental determination study of the temperature dependence of vapor pressure for ethyl myristate (ethyl tetradecanoate) is presented. There is limmited published data for this.

The aim is, first of all, evaluation of precision theoretical predictions they offer Ceriani-Meirelles method and in the same time complement the existing dataset.

The vapor-liquid equilibrium equipment VLE 50 bar and its main components are presented in Fig. 3.1.

The temperature dependence of vapor pressure for the ethyl myristate (ethyl tetradecanoate) has been measured with this equipment, on the temperature domain 150 to 220 °C. The choice of ethyl myristate to perform the determinations was done for economic reasons and due to its high concentration.

Prediction of the vapor pressure by Ceriani-Meirelles method

Ceriani-Meirelles method based on the contribution group (Ceriani si Meirelles, 2004), allows the prediction of the vapor pressure, using equation (3.2):

$$\ln P = \sum_k N_k \left(A_{1k} + \frac{B_{1k}}{T^{1.5}} - C_{1k}\ln T - D_{1k}T\right) + \left[M_i \sum_k N_k \left(A_{2k} + \frac{B_{2k}}{T^{1.5}} - C_{2k}\ln T - D_{2k}T\right)\right] + Q \qquad (3.2)$$

were:

P – vapor pressure (Pa); T - temperature (K); N_k - the number of groups k in the molecule; M_i - molecular weight of the component i;

A_{1k}, B_{1k}, C_{1k}, D_{1k}, A_{2k}, B_{2k}, C_{2k}, D_{2k} – parameters obtained from the regression of the experimental data; k – number of the groups of component i;

Q –correction term, calculated using equation (3.3):

$$Q = \xi_1 q + \xi_2 \qquad (3.3)$$

where:

$$\xi_1 = f_0 + N_c \cdot f_1 \qquad (3.4)$$

$$q = \alpha + \frac{\beta}{T^{1.5}} - \gamma \ln(T) - \delta T \qquad (3.5)$$

$$\xi_2 = s_0 + N_{cs} \cdot s_1 \qquad (3.6)$$

In the above expressions, these parameters are used:

N_c – number of carbon atoms in the molecule;

N_{cs} – number of carbon atoms of the alcoholic part;

f_0, f_1, α, β, γ, δ, s_0, s_1 – optimized parameters obtained by regression of the experimental data, given in Table 3.2.

The functional groups characteristic for the alkyl esters are: $-CH_3$, $-CH_2$ and -COOH. Table 3 shows the values of the group parameters, specific for the Ceriani-Meirelles method.

The vapor pressures dependence at different temperatures for ethyl myristate are shown in Fig. 3.3a, comparatively with the Ceriani-Meirelles predicted values.

The predicted values are in a fairly good agreement with the experimental measurements on the first interval of temperatures, the differences being significant at temperatures above 210 °C (the error is approximately 10% for 220 °C). The results are remarkable, considering the fact that, in Ceriani-Meirelles method, the calculation of the substance does not require specific parameters deduced experimentally prediction is generated.

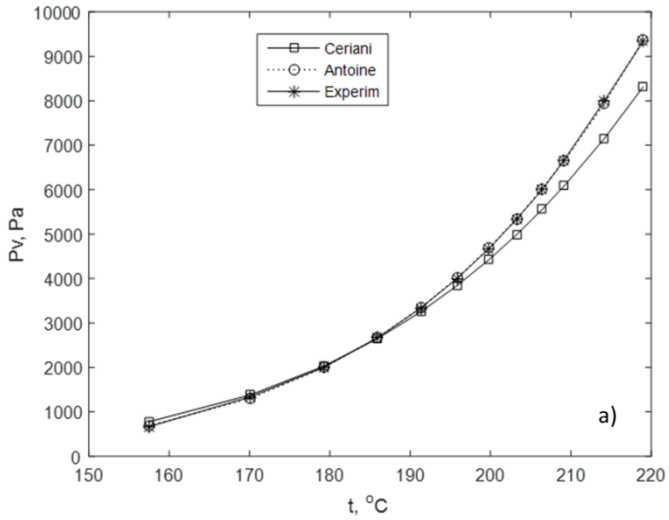

Fig. 3.3 Ethyl myristate vapor pressure vs temperature (Enascuta et al., 2017)

4. SEPARATION OF FATTY ACID ALKYL ESTERS

A separation procedure consisting in two steps was adopted (Figure 4.1). In a first stage, advanced vacuum distillation, preliminary separation was carried out at pressures between 2.4-5.8 Pa and temperatures between 150-170 ° C. In the scheme shown in Fig. 4.1, D1, D2, D3 and R1, R2 and R3, respectively, were noted the light and heavy fractions, resulting in three different distillation experiments (DP1-DP3), made on the same distillation column. In this first stage, D1-D3 the light fractions, rich in saturated fatty acid esters, were used as biodiesel and R1-R3 the heavy fractions rich in omega-3 fatty acid esters.

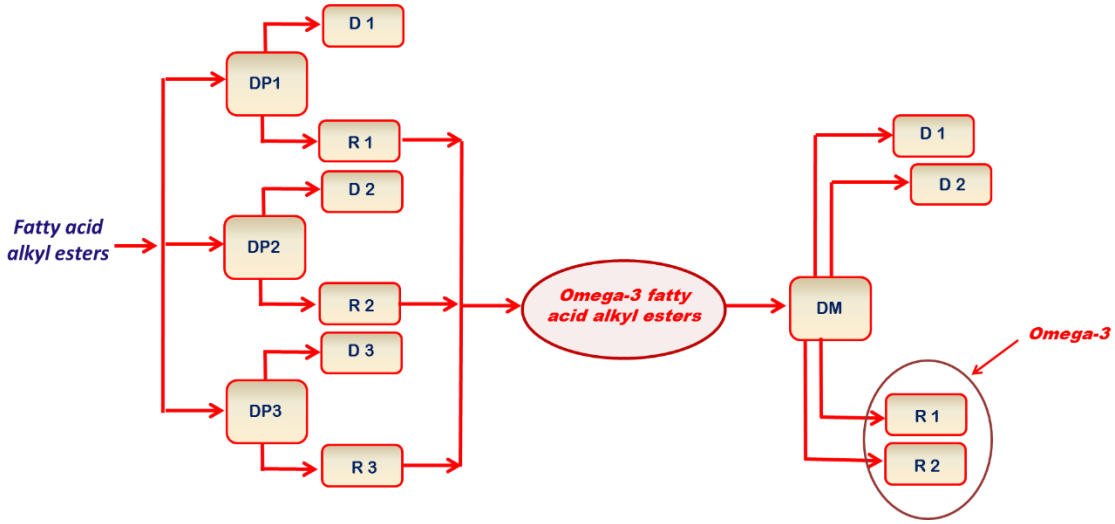

Fig. 4.1 The general scheme for the separation of fatty acids alkyl esters from fish oil

4.2 The separation step of omega-3 fatty acids ethyl esters from fish oil by vacuum distillation (first stage)

After establishing the operating conditions, omega-3 fatty acid esters of camelina oil (predominantly C18:3, ALA) and fish oil (C20:5, EPA, C22:6, DHA) was obtained by advanced separations on the vacuum distillation.

Table 4.2 Operating parameters for the separation of ethyl esters of fatty acids from fish oil by vacuum distillation

Parameters	**Exp. 1**	**Exp. 2**	**Exp. 3**
Pressure, Pa	4.6	2.4	5.8
T_{vapor}, ºC	156	157.4	165.2
Feed rate, g/h	111	109	467
Wiper speed (rot/min)	331	320	321
T_{oil} feed, ºC	85	85	85
T_{oil} evaporator, ºC	195	195	205
Tcondenser, ºC	33	32	32
Omega-3, feed composition, % mass	47.75		
Omega-3, light fraction composition, % mass	27.43	30.28	33.33
Omega-3, heavy fraction composition, % mass	62.93	69.04	76.31

Three experiments were carried out starting from the same raw material, modifying the residual flow and the heating oil temperature. The operating parameters used are described in Table 4.2.

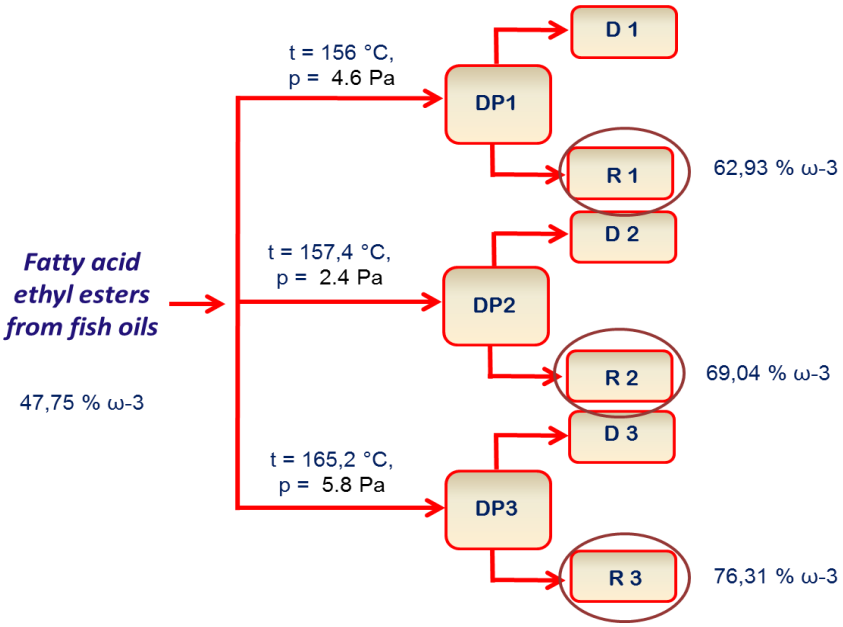

Fig. 4.5 The separation scheme of fatty acids ethyl esters from fish oil by vacuum distillation

Figure 4.5 describes the separation scheme for obtaining the heavy fractions rich in omega-3 fatty acids. It can be seen that, starting from a concentration of the 47.75% (mass) feed mixture, could be obtained heavy fractions with high content of polyunsaturated fatty acids up to 76% by changing the feed and residuu rate. Changing this rates, both vapor temperature and working pressure have changed.

The heavy and light fractions composition are describes in Table 4.3.

Following the vacuum distillation of the ethyl esters from fish oil, resulted three heavy and three light fractions. In Figure 4.6 it can be noticed that with the increase of temperature the

omega-3 fatty acid content is also increased, which is significantly higher in the residue than in the distillate.

In Figure 4.7 it is shown that, from "omega-3" esters class only C16:3 (max. 30.2%) and C18:4 (max. 4%) are found in light fraction. The rest of the omega-3 compounds are found only in the heavy fraction. All compounds of saturated or unsaturated C14-C18 are in high concentration in the light (distilled) fraction.

4.3 The separation step of omega-3 fatty acids ethyl esters from fish oil by molecular distillation (second stage)

The heavy and light fractions composition are describes in Table 4.6.
The saturated fatty acid ethyl esters with small chains of carbon atoms (C14-C16) are found in very small amounts in the heavy fraction, concentrating predominantly in the light (distilled) fraction.

Table 4.5 Operating parameters for the separation of ethyl esters of fatty acids from fish oil by molecular distillation

Parameters	**Exp. 1**	**Exp. 2**	**Exp. 3**	**Exp. 4**	**Exp. 5**
Time, min	15	29	47	62	82
Pressure, Pa	0.18	0.24	0.24	0.17	0.21
Tvapor, ºC	90	90	100	100	110
Feed rate, g/h	268.00	372.86	446.67	320.00	702.00
Wiper speed (rot/min)	311				
T_{oil} feed, ºC	85				
Tcondenser, ºC	22				
Omega-3, feed composition, % mass	71.41				
Omega-3, light fraction composition, % mass	48.22	43.79	44.86	51.27	53.23
Omega-3, heavy fraction composition, % mass	80.13	74.9	78.9	87.47	84.49

The polyunsaturated fatty acid ethyl esters from heavy fraction (residue) were efficiently concentrated, reaching concentrations up to 87%. The best results were obtained at a temperature of 100 ° C and a pressure of 0.17 Pa.

4.4 Mathematical modeling of the separation process with molecular distillation

4.4.1 Molecular distilation modeling based on physicochemical principles of the process

As presented in the first chapter, for molecular distillation separation process, there has been developed different important theoretical models. Starting from the method proposed by *Martinez si colab., (2011),* the aim is to continue to modeling the molecular distillation process by estimating the outcome of the operation conditions over the process. The experimental results presented in sub-chapter 4.1 obtained using molecular distillation equipment, have been compared with the model predictons generated by Langmuir-Knudsen equations and with the

method based on a group contribution (Ceriani et.al., 2004) that is needed for estimating the components vapor pressure estimation.

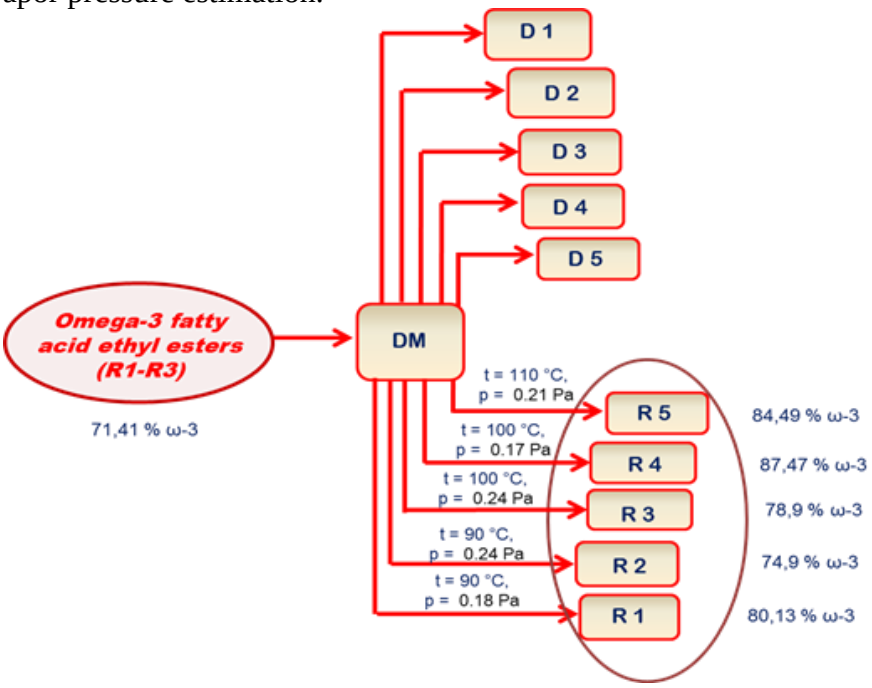

Fig. 4.15 The separation/concentration scheme of omega-3 fatty acids ethyl esters from fish oil by molecular distillation

Multiple process variable have been analized including their compositions.
Langmuir-Knudsen equation that describes evaporation molar rate on a surface unit (j_i) which is at the base of this model, is:

$$n_i = x_i p_i^0 \sqrt{\frac{1}{2\pi M_i RT}} \qquad (4.5)$$

Ignoring the combining phenomena of liquid phase on vertical direction, the component i debit variation on the liquid for separation (heavy fraction), related with the position along the evaporation area is described by the equation:

$$\frac{dW_i}{dz} = -\pi d_{ev} n_i \varphi, \quad i=1,..N_c \ , \ z=0, \ W_i=W_{i0} \qquad (4.6)$$

In (4.6) equation Martinez et al., (*2011*) have been introducing a corection factor, φ, that depends on working pressure and the debit of the mixture going into distilation. This coeficient have been introduce to correct some model imperfections, derived from the fact that Langmuir Knudsen model is overestimating the evaporated component flow (predicting the maximum theoretical evaporation flux), because it does not take into account the process reversibility (re-evaporation of a distilation part from the condensation area) and neither the efect of the molecules of the residual air existing in the column space. For φ computation, the authors have proposed the following:

$$\varphi = \frac{1}{\alpha P + \beta / D_m} \qquad (4.7)$$

In this α and β are paramenters that depend on the compound nature.

The equation system (4.6) has been integrated using constant temperature condition for evaporation surface, utilizing for n_i the expression (4.5).

The vapours pressure have been calculated with Ceriani-Meirelles method, represented in paragraph 3.1.1. For this purpose, the (3.2)-(3.6) relations have been used, in which the groups and group contributions from Table 3.2 have been considered.

Molar debits that were formed on condensation surface (for distilation), D_i, are calculated, ignoring the loss of vapors in the molecular distilation surface, from:

$$D_i = W_{i0} - W_i \qquad (4.9)$$

Integration of the diferential equation system (4.6) has been numerically perfromed, using the *ode45* function from scientifical computation microlanguage *Matlab.*

The results are presented in the figures (4.22) – (4.24), like a mass flow evolution and mass fraction along the column evaporation surface, for the two out of 5 experiments from the previous paragraph (experiments 1 and 4). On this charts, the values at the exit of the column, for debit and concetration, are compared with the values experimentally obtained for the same sizes.

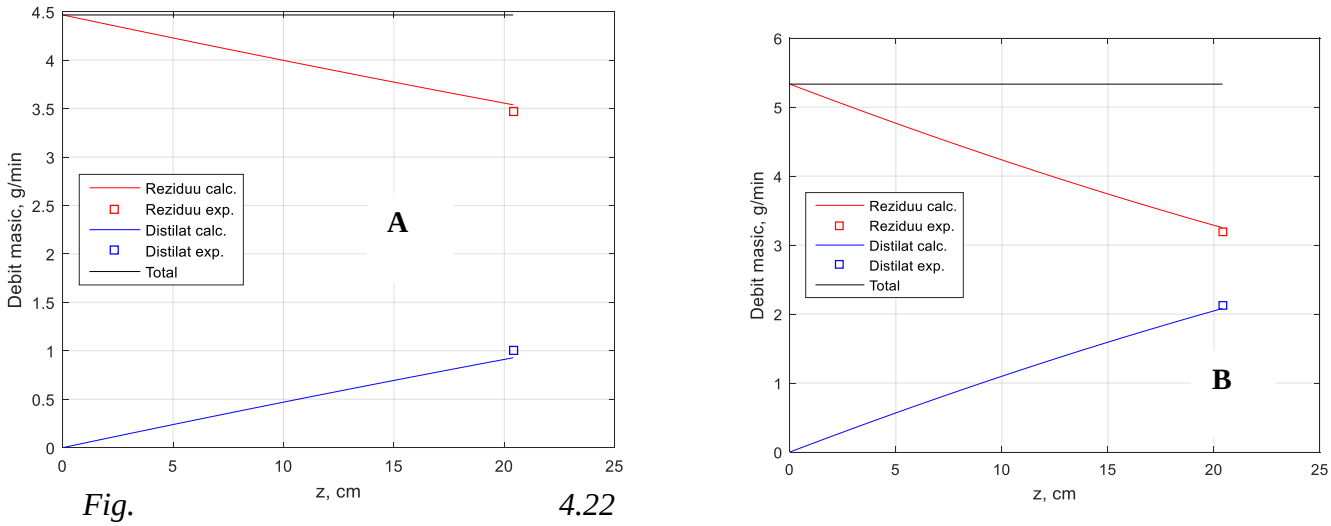

Fig. 4.22 Evolution of the mass debits for heavy fraction (reziduu), light fraction (distiled) and total, A) experiment 1; B) experiment 4

Figure 4.22 describes the calculated mass and distiled debits evolutions and residual respectively, along the distilation column, for the 1 and 4 experiments. The values at the exit of the equipment, are equal with the ones from experiment. Figures 4.23 and 4.24 shows the mass fractions evolution for the components in light fraction and heavy fraction, for both experiments. For both experiment 1 and experiment 4, the mass fractions computed values for C20:5 (EPA) and C18:1, are diverging from the experimental values at the exit of the equipment, being over-evaluated. In the heavy fraction, the maximum deviations for computed values are for C20:5 and C22:6 ester respectevely.

The observed modeling errors can be from simplified hypotheses for the model, from vapour pressure calculation erros and from eperimantal mesurements of the compounds, for which the errors were analysed above.

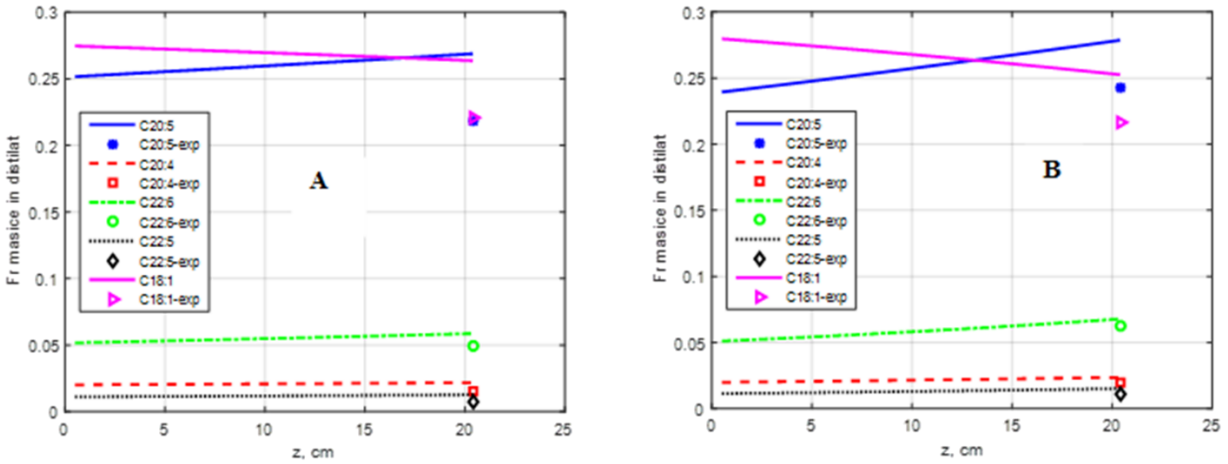

Fig. 4.23 Concentrations (mass fractions) in light fraction (distiled), A) experiment 1; B) experiment 4

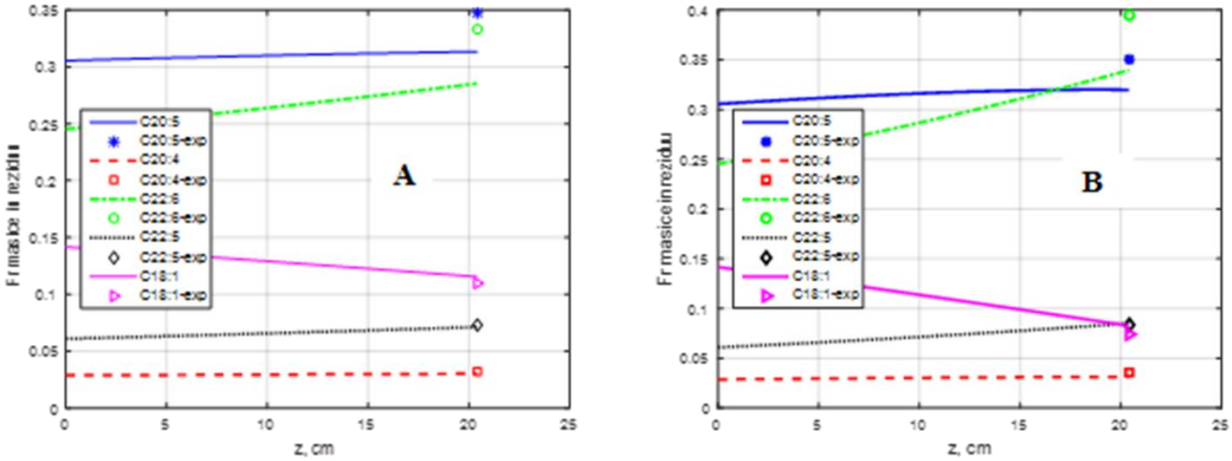

Fig. 4.24 Concentrations (mass fractions) in heavy fraction (residual), A) experiment 1, B) experiment 4

4.4.2 Modeling and optimizing the molecular distillation process using artificial neural networks (ANN)

For the molecular distilation column, we will consider a artificial neural network architecture that contains four input neurons: tempertature T, feed flow D, saturate fraction composition, FS (C18:0), and compositions of unsaturated compounds fractions FN, (C18:1, C18:2, C20:1, C20:4, C22:5). For hiden layer we will use in first case 1 neuron and in second case 2 neurons, and as output neurons we use only mass fraction of compounds omega-3, Fω-3 (C20:5 EPA si C22:6 DHA). With ω_{11}... ω_{24} have been used for averages. There haven't been used more hidden neurons due to limmited number of known data.

The obtained architectures for molecular distilation column are presented in Figure 4.25.

The Table 4.8 contains the values of the data used as input in network structure. The feed flow is between 200-400 g/h, and operating temperature is between 95-145 °C.

The separation process is influenced by a large number of factors. Multiple optimal variables are indentified such as: temperature, feed flow and compositions of fatty acid esters fractions.

Based on the chosen architecture for estimating molecular distillation process needed by distilation column, we have used *Regstats* din *Matlab R2015a* program.

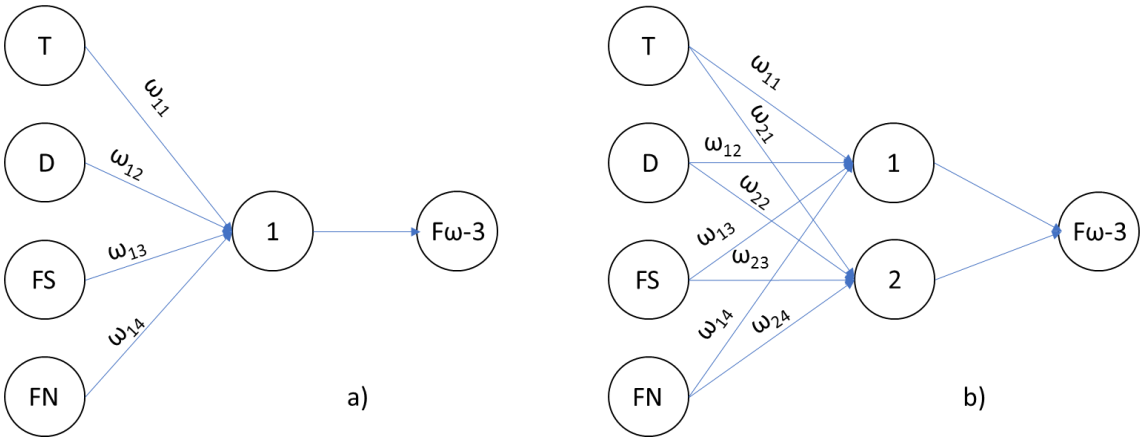

Fig. 4.25 Artificial Netural Network Architecture for studied process
(a) one hidden neuron, (b) two hidden neurons

The architectures used in this study with one and two hidden layers, are summarized in Table 4.

From Figure 4.25a, it can be observed that predicted data from artificial neural network with 2 hidden neurons are alighned with experimental data. In the case of the network with one hidden neuron (Fig. 4.25b) the results are satisfactory, and can be further improved.
The analysis of the resulted data are described in Tabel 4.10 and on the two regression graphics.

In Figure 4.25 are presented parity graphics for the two networks.

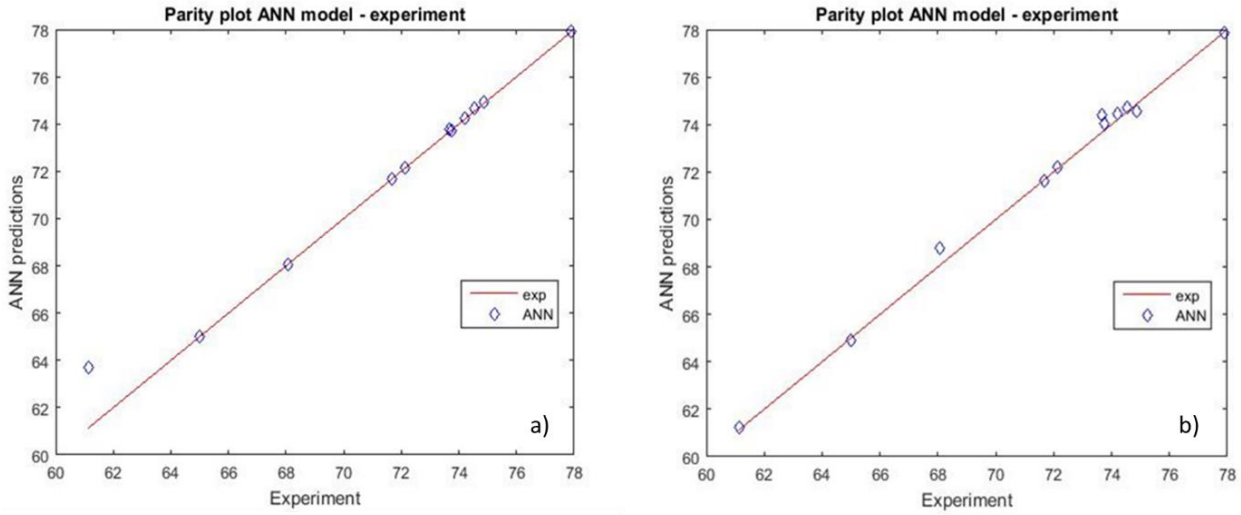

Fig. 4.25 Comparison of model-experiment data for molecular distilation process
a) one hidden neuron, b) two hidden neurons

Table 4.10 Optimal value estimated by the model

No.	Hidden neuron	D	T	FS	FN	Fω-3	R^2
1	1	180	81	0.79	19.09	82.35	0.99126
2	2	402.21	81	0.79	19.1	87.36	0.99938

where: R^2 – determination coeficient.
From both Figure 4.25a and Tabel 4.11 it can be observed that the model predicted results for entire studied domain are consistent with experimental data. The only differences are on experiments 8 and 9 where model predicted values are slightly higher than the experimental ones.

5. GENERAL CONCLUSIONS

The omega-3 fatty acid alkyl esters are thermolabile substances, with very low volatility and boiling points relatively close. One of the widespread separating techniques of this compounds group from the complex mixtures is advanced vacuum distillation and molecular distillation.

The research carried out within this thesis focused on: (i) the extraction of oils rich in omega-3 compounds, from microalgae and fish wastes and their purification; (ii) the preparation by transesterification of fatty acid esters rich in omega-3; (iii) determination of the properties of these esters useful in the development of procedures for the separation by distillation; (iv) development of the procedures for obtaining the concentrated omega-3 fatty acid esters, through the combined operations of vacuum distillation and molecular distillation; (v) development of mathematical models useful in designing and optimizing processes for the separation of omega-3 fatty acid esters by molecular distillation.

The studies have led to the following main conclusions:

1. Microalgae and fish waste derived from its normal processing, for food purposes are interesting sources to obtain the oils rich in omega-3 fatty acid esters. We have developed and tested the obtaining procedures, by extracting some oils rich in triglycerides of polyunsaturated fatty acids from fish waste and microalgae cultivated in ICECHIM Bucharest laboratories. The processes includes an extraction step followed by a purification step of the crude oil. The composition analyzes revealed higher concentrations of omega-3 fatty acid esters in fish oil (~ 35% in fish oil compared to ~ 20% in the algae).

2. As fish oils have a relatively high acidity (acid index ~ 17), a study of the esterification step of the free acids contained in these oils was carried out. Esterification was performed with ethanol on a solid acid catalyst of the SO_4^{2-} / SnO_2 -ZrO2 type using an simulation procedure of the experiments (D-optimal program). The obtained results allowed the deduction of a nonlinear statistical model which expresses the dependence of fatty acid conversion on the working temperature and the molar ratio of alcohol / free fatty acids, respectively.

3. A transesterification study of the low acidity oils obtained after the previous step with ethanol in the presence of a basic catalyst (KOH) was performed. The proposed process consists in two transesterification stages, with the intermediate separation of glycerol, which ensures practically total conversion of triglycerides from oils. The composition analysis of the obtained esters mixtures, are performed by chromatographic methods combined with mass spectrometry (GC-MS).

4. Have been evaluated the main methods proposed in the literature for assessing the physical properties of unsaturated fatty acids esters (vapor pressure / boiling points, densities, viscosities). To calculate the vapor pressures of these classes of the compounds, the Ceriani-Meirelles method based on group contributions, is used. The method was tested, by comparison with experimental data obtained in the current study, for a accessible purely ester (ethyl myristate). The results have shown an acceptable predictive capacity of this method, which is still used in the modeling of the molecular distillation process.

Estimating the kinematic densities and the viscosities of the fatty acids alkyl esters mixtures resulting from the oil transesterification process was performed using the AspenPlus, Simulis and CosmoTherm computing programs. The comparison with the literature data revealed the suitability of these predictions, using AspenPlus and Simulis, especially.

5. In order to obtain the omega-3 ester concentrates, a two step separation procedure was adopted, a first stage of distillation under high vacuum (~ 1-5.4 Pa), followed by a second stage of vacuum molecular distillation (~ 0.1 Pa). As typical results, it is mentioned that, starting from

a concentration of 47.75% mass feed of the omega-3 esters, enriched fractions were obtained in these esters with concentrations up to 76%. Further, in the molecular distillation stage, the concentrations of polyunsaturated esters reached up to 87% (mass). The overall yield in 'omega-3' unsaturated ester concentrates, calculated in relation to purified fish oil, is about 33%, not considering the use of the light fractions resulting from the two distillation stages (vacuum and molecular).

6. The usage value of the omega-3 acids is lower than those of glycerol esters with these unsaturated acids (triglycerides of omega-3 fatty acids). Based on this consideration, a transesterification study of the ethyl esters obtained in the concentrates resulting from the previously described separation steps with glycerin ('retro' transesterification) was carried out. By conducting the reaction under vacuum (25-30 mbar pressure) with continuous elimination of the reaction product (ethanol) in the basic catalysis (KOH) at 125-135 °C for 6-8 hours in a discontinuos sistem under stirring, the total conversion of the triglycerides are obtained.

7. The study of the mathematical modeling and the simulation of the molecular distillation operation consists in the application of two methods, an analytical one based on conservation equations and another empirical, based on the Artificial Neural Network (RNA) algorithm. In the first method, the model proposed by Martinez et al (2011), coupled with the Ceriani - Meireilles (2004) method for assessing the vapor pressure of the components, was adopted. The resulting model ensures a satisfactory precision of the prediction of molecular separation by leading to modeling errors of the same magnitude order as those identified in the experimental data. The RNA modeling method, performed in two variants of the network structure, leading to a very good correlation to molecular distillation process variables. The elaborated RNA procedure allows predictions of separation and optimization of working conditions in the experimentally studied of the temperature, pressure, and composition.

Proposals to continue the research

The experimental results of the research elaborated within this thesis open new ways to valorization of the raw materials rich in polyunsaturated fatty acids

Among the future directions of research to be approached can be summarized:

- Continue the studies regarding improving the process of the oil recovery from fish waste;
- The recovery study of the valuable components (omega-3 esters) from the light fractions obtained by vacuum distillation and molecular distillation
- To improve the estimation methods of the physical properties of these esters
- Elaboration of more complex operation mathematical models for molecular distillation, considering the variations of the temperature and the flow rates in the liquid films formed on the evaporation and condensation surfaces.

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