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PhD Thesis

ω-polyunsaturated fatty acid derivatives production from natural products

(PhD Thesis Summary)

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TABLE OF CONTENTS

Keywords: ω-PUFA methyl esters, *Camelina sativa* oil, FAME vapor pressure, FAME viscosity, FAME density, experimental measurements, data prediction, group contributions, oil deacidification, optimization, Box-Behnken design, response surface, molecular distillation, high vacuum distillation

INTRODUCTION

Omega polyunsaturated fatty acids (ω-3 PUFA and ω-6 PUFA) consumption is associated with different beneficial effects on human health. There are four types of ω-3 PUFA: eicosapentaenoic acid (C20:5), docosapentaenoic acid (C22:5), docosahexaenoic acid (C22:6) and linolenic acid (C18:3). The first three fatty acids are found in marine sources such as fish and algae and the last one is found mainly in vegetable sources, such as vegetable oils. The group of ω-6 PUFA includes linoleic acid (C18: 2) which is found only in vegetable oils. ω-PUFA fatty acids are characterized by three features: they cannot be synthesized by the human body in enough quantity, the insufficient amount causes deficiency symptoms, and the deficiency symptoms can be corrected if the necessary amount of nutrients is administered through the diet.

For this study, a vegetable oil obtained by extraction from the seeds of the *Camelina sativa* plant was chosen as raw material because it has different advantages such as a balanced profile of ω-PUFA fatty acids (52-56%) and an advantageous purchase price. Camelina oil is less stable than other oils and for this reason is not included in the list of edible oils. Long time storage causes oxidation and increases oil acidity. Oil acidity is given by the content of free fatty acids (FFA). The FFA content is one of the most important factors that influence the oils quality and it is recommended to have a low value. Conventional methods such as alkaline treatment and washing with significant amounts of water to remove the soap are not recommended due to the large oil losses and the resulting large amount of wastewater.

The main objective of the PhD thesis is to obtain concentrates of ω-PUFA fatty acids esters (omega polyunsaturated fatty acid derivatives) starting from a vegetable raw material (*Camelina sativa* oil), using advanced separation methods. These concentrates have an antioxidant effect and can be used in the pharmaceutical industry to obtain concentrated food supplements.

The scientific research was set in the following directions:

1. FAME properties for vacuum ranges

FAME mixtures obtained by transesterification have a complex composition and their properties are very difficult to be measured experimentally due to the thermolabile character. For the separation processes which were investigated in this paper, properties such as the boiling temperature of the mixtures and density/viscosity of the mixtures are very important and depend on the composition and system pressure. Conducted research:

a) Vapor pressures of pure fatty acid esters
    - Experimental measurements of vapor pressures for methyl linoleate and ethyl oleate in the pressure range 0.1-30 mbar;
    - Formulation of different models for estimating vapor pressures by experimental data regression (Clapeyron, Antoine, Riedel, extended Antoine) and validation with literature data;
    - Model formulation for estimating vapor pressures by Ceriani-Gani-Lu method, based on group contributions and validation with literature data;
    - Formulation of a model for estimating pressures by correlations based on critical properties of the components (corresponding states principle) and validation with literature data;

b) Viscosity of FAME mixtures
    - Experimental measurement of the viscosity for 16 FAME mixtures with different composition in the temperature range 298.15-363.15 K and atmospheric pressure.
    - Models formulation for estimating viscosities by GCVOL method proposed by Elbro, 1991, based on group contributions and validation with data from the literature;

c) Density of FAME mixtures
    - Experimental measurement of the density for 16 FAME mixtures with different composition in the temperature range 298.15-363.15 K and atmospheric pressure.
    - Model formulation for estimation density by Ceriani method, based on group contributions and validation with data from the literature;

Experimental research results: Regression models for estimating the vapor pressures of fatty acid esters, density and viscosity regression models of fatty acid ester mixtures.

2. Process for treating degraded oils by modern techniques (molecular distillation)

The experimental research aimed to find a method for treating oils with different acidity (freshly pressed or with high acidity) by removing the free fatty acids which can influence the transesterification reaction by forming soaps. Conventional methods (alkaline treatment and water washing) are disadvantageous due to high oil losses and large amount of wastewater.

Experimental research results:

a) Experimental research on the operating parameters (evaporation temperature, wiper speed and feed rate) for camelina oil deacidification by molecular distillation and their effect on process efficiency. The distillation products were analyzed by GC-FID chromatography (each sample was analyzed five times and the results were presented as the average value ± standard deviation).

b) Studies on the optimization of vegetable oil deacidification by molecular distillation using the response surface method. A three-factor and three-level Box-Behnken design (BBD) was formulated based on experimental deacidification data. Three regression models were formulated for the FFA concentration, the deacidification efficiency and the triglycerides concentration in the deacidified oil, as well as the effect of evaporation temperature, wiper speed and feed rate on these responses. The analysis of response surfaces and desirability function determined the optimal parameters for this process: evaporation temperature of 173.5°C, wiper speed of 350 rpm and feed rate of 2 mL/min, to reduce the FFA concentration from 7.67% to 1.007%, with high deacidification efficiency (92.167%) and high TAG concentration (93.360%).

Value-added products: deacidified *Camelina sativa* oil with 0.73% FFA and 95% fatty acid concentrate.

3. Process for obtaining ω-PUFA methyl ester concentrates by modern separation methods (molecular distillation and advanced vacuum distillation)

ω-PUFA methyl esters obtained by transesterification of deacidified camelina oil with methanol (C18:2, C18:3) are difficult to separate because they have close boiling points and are thermosensitive components. At very low pressure (high vacuum conditions), the boiling temperatures drop below the degradation limit, but the differences between the esters boiling points are very small (1°C) and cannot be separated by classical distillation methods. Another drawback is that the physicochemical properties of these components cannot be easily measured under these conditions. The research activity was aimed on finding an efficient method for separating ω-PUFA methyl esters. Molecular distillation is a distillation technique based on the separation of vaporized molecules which travel a distance less than or equal to the average free path (which depends on the molecule volume) and condenses when it reaches a cold surface.

The operating conditions were determined by experimental trials, starting from values calculated by mathematical methods.

Experimental research results:

a) Obtaining the FAME mixtures for the experimental research by transesterification of deacidified camelina oil in basic homogeneous catalysis;
b) Obtaining ω-PUFA mehyl esters concentrates by advanced separation methods - molecular distillation and high vacuum distillation ($p \leq 1Pa$). Four separation schemes with different distillation steps and optimal separation parameters were formulated (obtained by experimental trials, starting from the properties calculated with the regression models);
c) Distillation products characterization by GC-MS chromatography (each sample was analyzed five times and the results were presented as the average value ± standard deviation);

Value-added products: 73.32% ω-PUFA ME concentrate with a yield of 60.04%, and two secondary products - 96.10% methyl palmitate concentrate and 89.20% methyl eicosenoate-methyl docosenoate concentrate.

The four separation schemes of ω-PUFA methyl esters are shown in fig.1-4.

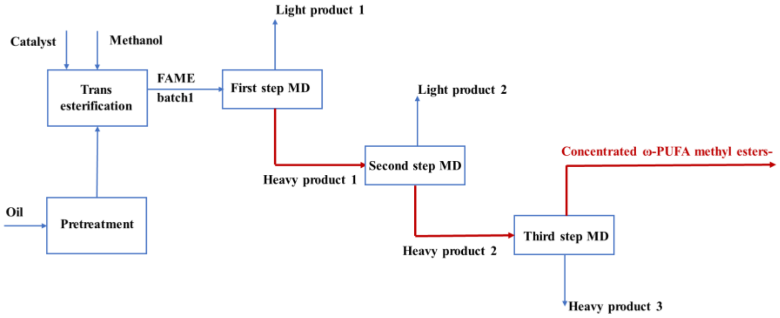


Fig. 1. Three steps model MD scheme (63.09% ω-PUFA ME concentration with a yield of 53.87%)

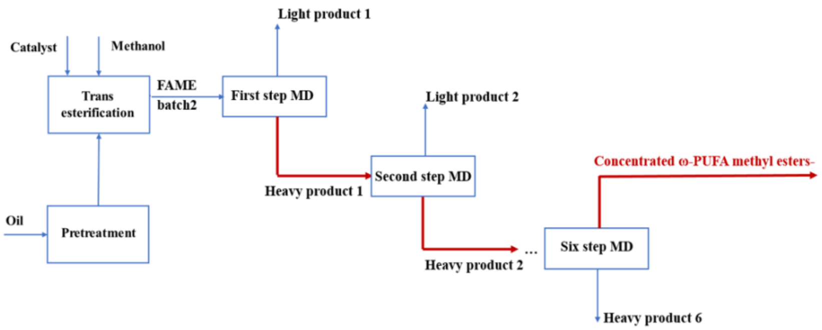


Fig. 2. Six steps model MD scheme (61.80% ω-PUFA ME concentration with a yield of 40.87%)

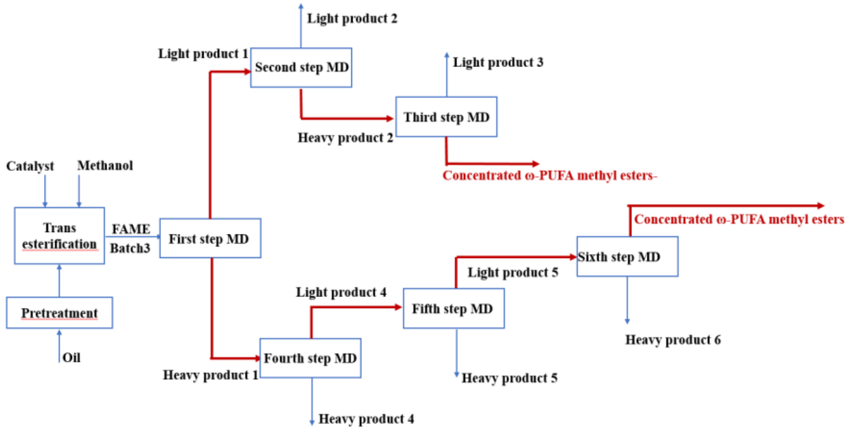


Fig. 3. Combined steps model MD scheme (62.04% ω-PUFA ME concentration with a yield of 62.61%)

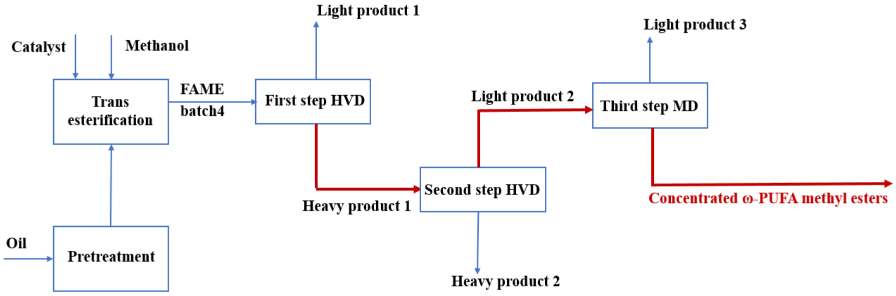


Fig. 4. HVD combined with MD scheme (73.32% ω-PUFA ME concentration with a yield of 60.04%)

The PhD thesis is devided in six chapters:

Chapter 1 presents the original contributions.

In *chapter 2*, the properties of FAME obtained from *Camelina sativa* oil were determined: vapor pressure of pure compounds and viscosity/density of 16 FAME mixtures.

Vapor pressures. The vapor pressures of methyl linoleate and ethyl oleate were measured at low pressure (0.1-30 mbar), using a vapor lichid equilibrium equipment. For validation, predicted vapor pressures by different methods were used: correlation (Clapeyron, Antoine, Riedel and extended Antoine), group contribution (Ceriani-Gani-Lu) and corresponding states (Reynes and Thodos with eight combinations of critical properties and normal boiling point, from different databases). Based on RMSD error and R^2 coefficient the best prediction method was choosen and applied to calculate de vapor pressures for all the compounds in the FAME mixture (C14:0, C16:0, C18:0, C18:1, C18:3, C20:1, C22:1)– coresponding state principal method, using prediction 2. Based on these, the boiling points of different FAME mixtures were calculated.

Viscosities and densities. Viscosities and densities of 16 FAME mixtures were measured in the range of 298-363K and atmospheric pressure. To validate the experimental data they were compared with data calculated by different group contributions methods. For the viscosities of FAME mixtures, Ceriani method was used to predict the viscosity of pure components. Using Grunberg-Nissan equation, the mixtures viscosities were determined, obtaining standard RMSD deviations between 0.4-8%. Also, for validating the experimental data, Constantinou and Gani database from Propred® together with Grunberg-Nissan equation were used. The obtained values were consistent with the experimental data, obtaining RMSD values between 0.4-3.7%. The GCVOL group contribution model was used to calculate the density of FAME mixtures, which predicted with small errors the experimental measurements (0.01-0.03%). Two databases implemented in Propred® (Marrero and Gani, Constantinou and Gani) and the linear mixing rule proposed by Kay were also used to estimate the densities of FAME mixtures. RMSD values between 0.05 and 0.15% were obtained.

Chapter 3 presents a study on the purification of *Camelina sativa* oil with an acidity index of 7.17 mg KOH/g oil and 7.67% FFA by molecular distillation. Experimental studies have shown a deacidification efficiency of over 90% and a FFA concentration in the heavy product of less than 1%. Different studies have been performed on the influence of certain operating parameters on oil quality. Three of these parameters (evaporation temperature, wiper speed and feed flow rate) influence the deacidification efficiency by 36%. At 200°C, 300 rpm and 2 mL/min, 90.5% of the FFA was removed and the TAG concentration increased by ≈10% in the refined oil compared to the crude oil.

Optimal operation conditions for FFA separation by molecular distillation were investigated using the Box-Behnken method with three factors and three levels. Based on statistical techniques, a second-order polynomial model was formulated to describe the system, effects and interactions between factors and the model was solved by minimizing/maximizing the response variables. To avoid extreme operation conditions, evaporation temperatures between 160-180ºC, wiper speeds between 250-350 rpm and feed rates between 2-6 mL/min were chosen for the optimization. In order to reduce FFA concentration from 7.67% to 1.007%, with high deacidification efficiency (92.167%) and high TAG concentration (93.360%) it is necessary that the molecular distillation be carried out under the following operating conditions: evaporation temperature of 173.5ºC, wiper speed of 350 rpm and feed rate of 2 mL/min. Optimal responses showed a desirability of 0.9826. In addition, two other experimental trials were performed under optimal operating conditions and the results were close to those predicted by the model.

In *chapter 4*, four strategies for ω-PUFA ME separation were presented. ω -PUFA are valuable compounds used in the pharmaceutical (food supplements) and food industries. They are found in mixtures with saturated and unsaturated fatty acids in vegetable, fish or algal oils and separation by traditional methods is difficult to achieve. To increase the ω-PUFA volatility, a preliminary step of transesterification of camelina oil with methanol was performed, obtaining ω-PUFA ME. Four strategies based on molecular distillation and high vacuum distillation were studied: TSM-MD, SSM-MD, CSM-MD, HVD-MD. The TSM-MD strategy led to a separation yield of the ω-PUFA ME of 53.87%, obtaining 449.39 g main product of 63.09% concentration. Keeping the same principle but increasing the number of MD steps, the second strategy (SSM-MD) led to a separation yield of 40.87% and 338.81 g of main product was obtained with a concentration of 61.80%. In order to recover a higher amount of ω-PUFA ME, the third separation strategy (CSM-MD) was proposed and 62.61% of ω-PUFA ME were recovered, obtaining 536.52 g main product of 62% concentration. The last strategy consisted of two HVD steps, where in the first stage the light component was removed and in the second stage the heavy components. The resulting product, highly concentrated in ω-PUFA ME, was then purified by molecular distillation and 316.91 g main product of 73.32% concentration with a separation yield of 60.04% was obtained.

Chapter 5 presents the conclusions of the scientific research.

In *Chapter 6* the bibliographical references are presented (143 references).

CHAPTER 2

ESTIMATION OF FATTY ACID METHYL ESTERS PROPERTIES

This chapter presents the results of the regression models used to predict FAME properties:

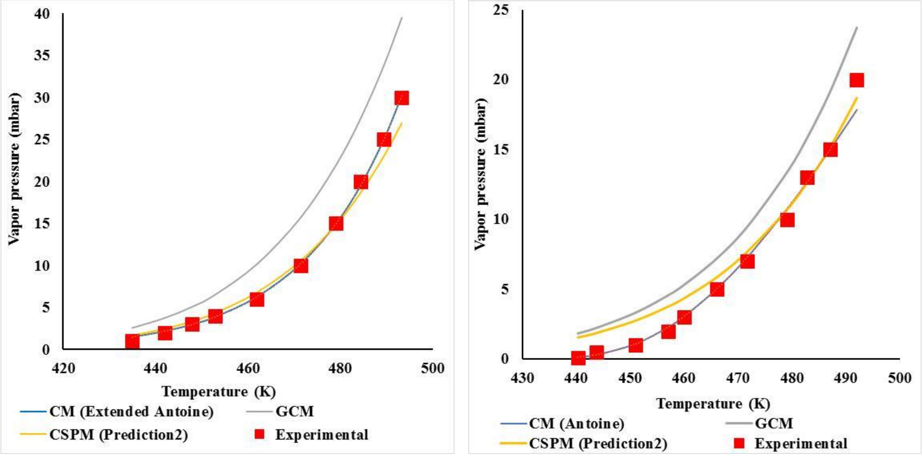
- vapor pressure of pure components;
- viscosity of the FAME mixtures;
- density of the FAME mixtures;

Fatty acid esters with a similar structure to those found in the composition of camelina oil were used for the experimental determinations. The vapor pressures for methyl linoleate (C18:2) and ethyl oleate (C18:1) were measured at low pressure (1-30 mbar for methyl linoleate and 0.1-20 mbar for ethyl oleate) using a vapor-liquid equilibrium equipment *Fischer® Labodest® VLE 50 bar*. The experimental data were compared with predicted vapor pressures by three methods:

- *correlation based on experimental data* (Clapeyron, Antoine, Riedel and extended Antoine models);
- *group contribution* (Ceriani-Gani-Lu method);
- *corresponding state principal* (Reynes și Thodos model in which eight different combinations of critical properties and normal boiling point were used);

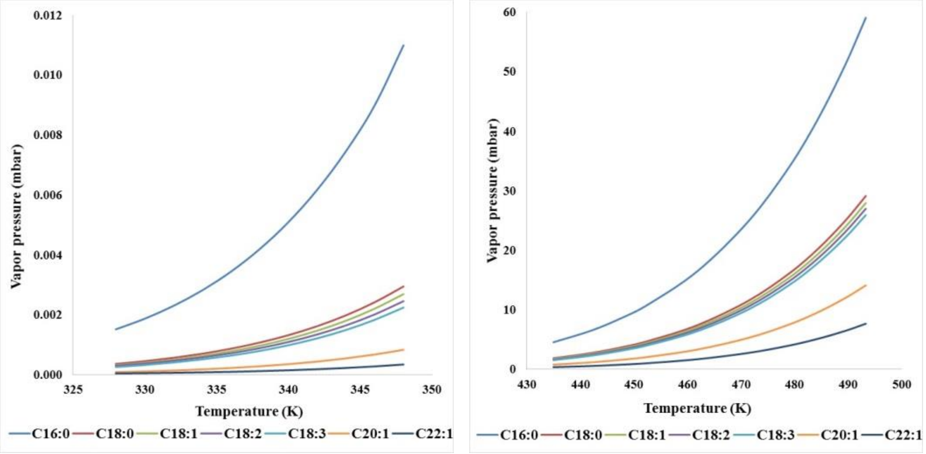
Estimated vapor pressures were compared with the experimental data, using standard deviation RMSD and the correlation coefficient R^2. The vapor pressures predicted with the corresponding state principle model were the closest to the experimental data for the two esters (RMSD of 1.30 for methyl linoleate, 1.18 for ethyl oleate respectively). Fig.5a shows the vapor pressures of methyl linoleate and Fig.5b shows the vapor pressures of ethyl oleate. The obtained regression model were used to estimate the vapor pressures for other methyl esters (C14:0, C16:0, C18:0, C18:1, C18:3, C20:1, C22:1), for two pressure ranges: 0.1-10 Pa (pressure range for separation by molecular distillation) and 5-30 mbar (pressure range for separation by high vacuum distillation). Fig. 6 shows the variation of vapor pressure for seven fatty acid esters obtained by camelina oil transesterification in the mentioned pressure ranges.

Measurements of densities and viscosities for 16 FAME mixtures obtained from molecular distillation operations (in the temperature range of 298.15-363.15 K and atmospheric pressure) were performed to obtain regression models. These mixtures contain the same type of fatty acid esters, but have different compositions. Regression models were formulated based on experimental data, and also prediction models based on group contributions.



a) b)

Fig. 5. Experimental vs predicted vapor pressure by: correlation methods (CM), group contribution method (GCM), corresponding state principal method (CSPM) for a) methyl linoleate; b) ethyl oleate;

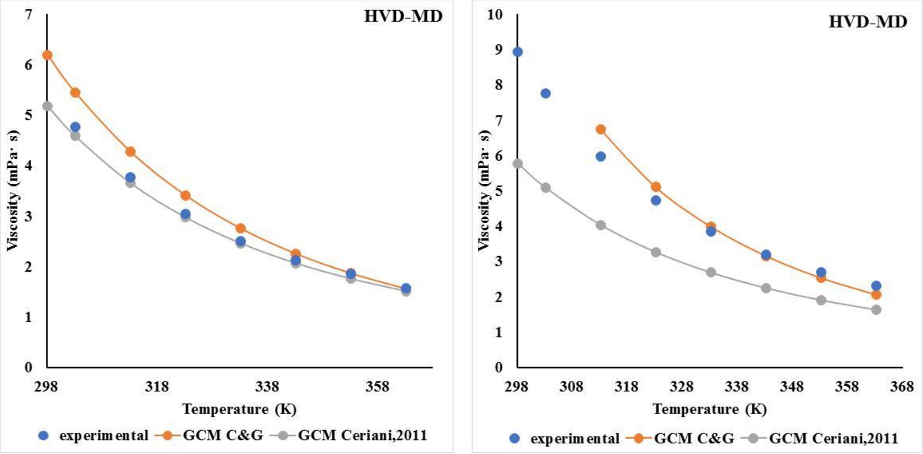


a) b)

Fig. 6. Vapor pressures estimation for fatty acid esters with CSPM model: a) pressure range 0.1-10 Pa, b) pressure range 1-30 mbar

The obtained results were compared with experimental data depending on the standard deviation (RMSD between 0.4% and 8%). Standard deviation increases with the increasing concentration of heavy compounds in the mixture. Also, the Ceriani prediction method led to higher standard deviations in case of heavy components. Fig.7 shows the viscosity variation of two mixtures of esters with predominant content of light and heavy compounds, predicted with the two methods and compared to the experimental data. GCVOL group contribution method proposed by *Elbro, 1991* was used to calculate the density of FAME mixtures and Marero-Gani

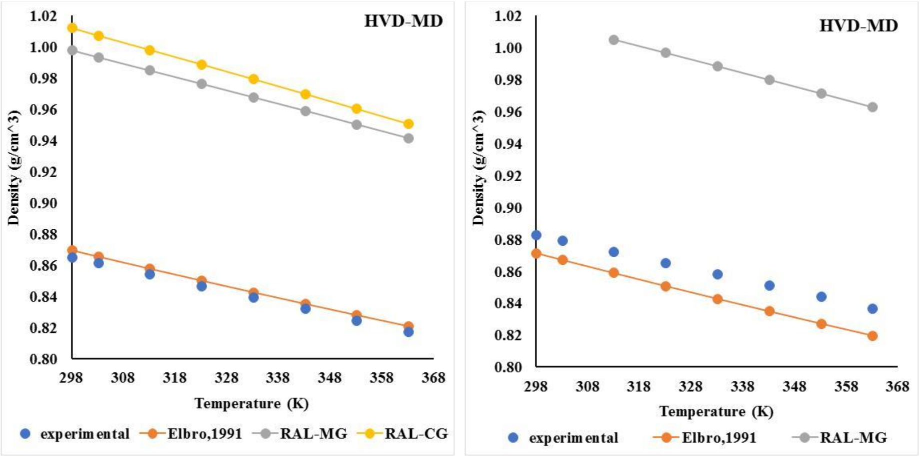
(*RAL-MG*) and Constantinou-Gani (*RAL-CG*) methods were used to predict the density of pure components, while the linear mixing rule proposed by Kay was used to calculate the density of ester mixtures.



a) **b)**

Fig. 7. Viscosity estimation for fatty acid esters: a) mixture of light compounds b) mixture with heavy compounds

The results were compared with experimental data, depending on the standard deviation (RMSD between 0.02% and 0.12%). Standard deviation increases with increasing concentration of heavy compounds in the ester mixtures. A small standard deviation was obtained for the densities predicted with Elbro model.



a) **b)**

Fig. 8. Density estimation for fatty acid esters: a) mixture of light compounds b) mixture with heavy compounds

Fig.8 shows the density variation of two ester mixtures with a predominant content of light and heavy compounds predicted with the two methods and compared to the experimental data.

CHAPTER 3

CAMELINA OIL PURIFICATION USING ADVANCED SEPARATION METHODS (MOLECULAR DISTILLATION)

In this chapter the deacidification process of degraded camelina oil was studied. Conventional deacidification methods, frequently used on industrial scale, include chemical and physical processes, each having different advantages and limitations. Deacidification by conventional methods always results in considerable oil losses and other valuable compounds (sterols, tocopherols and vitamins), but also with other problems in removing the soap which have a negative impact on the environment (large amounts of wastewater). These disadvantages can be corrected by alternative deacidification methods such as supercritical fluid extraction or molecular distillation.

3.1 *Camelina sativa* oil characterization

For the characterization of *Camelina sativa* oil the following determinations were performed, in accordance with European standards:

- SR EN Standard No. 660 - acidity determination

Tabel 1. Determination of *Camelina sativa* oil acidity

	sample 1	**sample 2**	**sample 3**	**sample 4**	**sample 5**
Oil (g)	4.917	4.838	4.957	5.121	4.965
V ethanol (mL)	25	25	25	25	25
V ethyl ether (mL)	25	25	25	25	25
V titration KOH sol. (mL)	1.20	1.20	1.20	1.25	1.20
I_A (mg KOH/g oil)	7.170	7.287	7.112	7.171	7.101
I_A average (mg KOH/g oil)	7.168				
SD	0.07				
RSD	1.03				

- SR EN Standard No. 14105 - determination of glycerides and fatty acids content

Tabel 2. Composition of *Camelina sativa* oil

	FFA	**MAG**	**DAG**	**TAG**
sample 1	7.92	0.89	6.00	85.19
sample 2	7.97	0.92	5.95	85.16
sample 3	7.45	0.82	5.67	86.06
sample 4	7.32	0.72	6.40	85.56
sample 5	7.70	0.82	6.04	85.44
average	7.67	0.84	6.01	85.49
SD	0.28	0.08	0.26	0.36
RSD	3.71	9.27	4.34	0.43

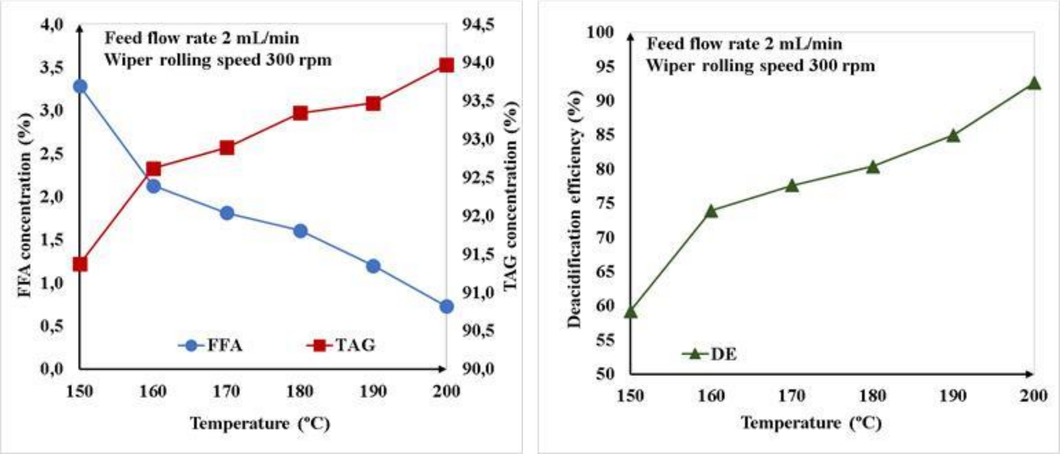
According to these standards, *Camelina sativa* oil has an FFA content of 7.67% and an acidity index of 7.17 mg KOH / g oil.

3.2.Experimental studies on *Camelina sativa* oil deacidification by molecular distillation

For the free fatty acids removal molecular distillation was used, an advanced separation method based on the evaporation of light molecules and their shifting to a cold surface where they condense. The mean free path of these molecules must exceed the distance between the evaporator and the condenser. The deacidification was performed with a KDL5 molecular distillation equipment manufactured by UIC GmbH, Germany. The operating conditions have been set as follows: evaporator temperature (150-200ºC), wiper rolling speed (250-350 rpm), feed flow rate (2-6 mL/min), pressure (0.1 Pa) and condenser temperature (19ºC). Experimental studies have been performed to determine the influence of operating conditions on free fatty acid content, deacidification yield and triglyceride content. Samples of light (fatty acids) and bottom product (deacidified oil) were obtained which were characterized by chromatographic methods.

Evaporation temperature

Molecular distillation experiments were performed for evaporator temperature variations in the range 150-200^{o}C, while the other parameters were maintained on a constant value. Fig. 9 shows the effect of evaporation temperature on the concentration of FFA and triglycerides (TAG), as well as on the deacidification efficiency.



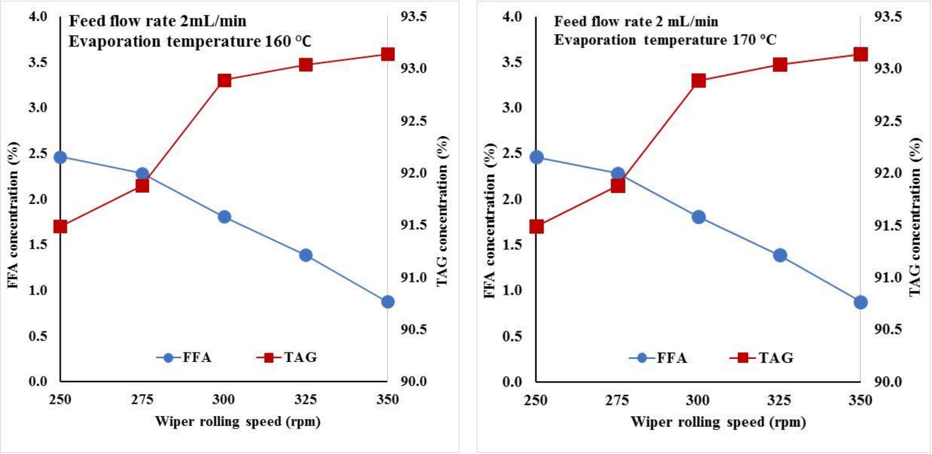
a) **b)**

Fig. 9. Effect of evaporation temperature on: a) FFA and TAG composition in deacidified oil, b) deacidification efficiency

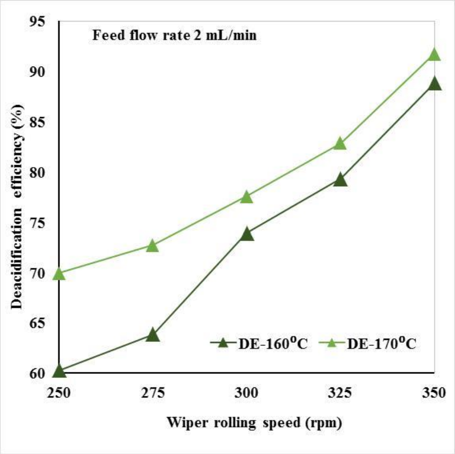
It has been noticed that increasing temperature, decreases the FFA content of degraded camelina oil and increases TAG concentration. In the deacidified oil, the FFA concentration decreased from 7.67% to 3.29% at 150ºC, respectively to 0.73% at 200ºC (Fig.9a). The process efficiency increased from 60% at 150°C to 92% at 200°C (Fig. 9b). It was noticed that at this temperature there were traces of oil degradation on the evaporator walls, therefore exceeding this temperature leads to changes in oil quality.

Wiper rolling speed

The wiper speed (UIC GmbH, Alzenau, Germany) has a significant influence on the molecular distillation separation process, mixing the liquid and dispersing it on the evaporator surface in a thin film. Fig. 10 shows the effect of wiper rolling speed on FFA and TAG content and on deacidification efficiency. These experiments allowed the evaluation of the stirrer effect on the volume of vapors that reach the condenser. The wiper rolling speed varied in the range of 250-350 rpm and the experiments were carried out at two evaporation temperatures: 160°C and 170°C. Increasing the wiper speed from 250 rpm to 350 rpm leads to a acidity decrease from 3.12% to 0.89% at 160ºC (fig.10a), respectively from 2.46% to 0.88% at 170ºC (fig.10a). The deacidification efficiency increased at high wiper speeds to 88.83% at 160ºC and 91.75% at 170ºC, respectively (fig.10c). The TAG concentration in the heavy product showed a moderate increase from 91.5% at 250 rpm to 93% at 350 rpm, with small differences for the two evaporation temperatures studied (fig.10a-b). The deacidification efficiency increased by 32.12% (at 160ºC) and 23.75% (at 170ºC) by increasing the wiper speed with 100 rpm due to the uniform distribution of the oil on the evaporator surface, facilitating evaporation. For efficient separation of FFA from camelina oil, the wiper speed must be higher than 300 rpm.



a) **b)**



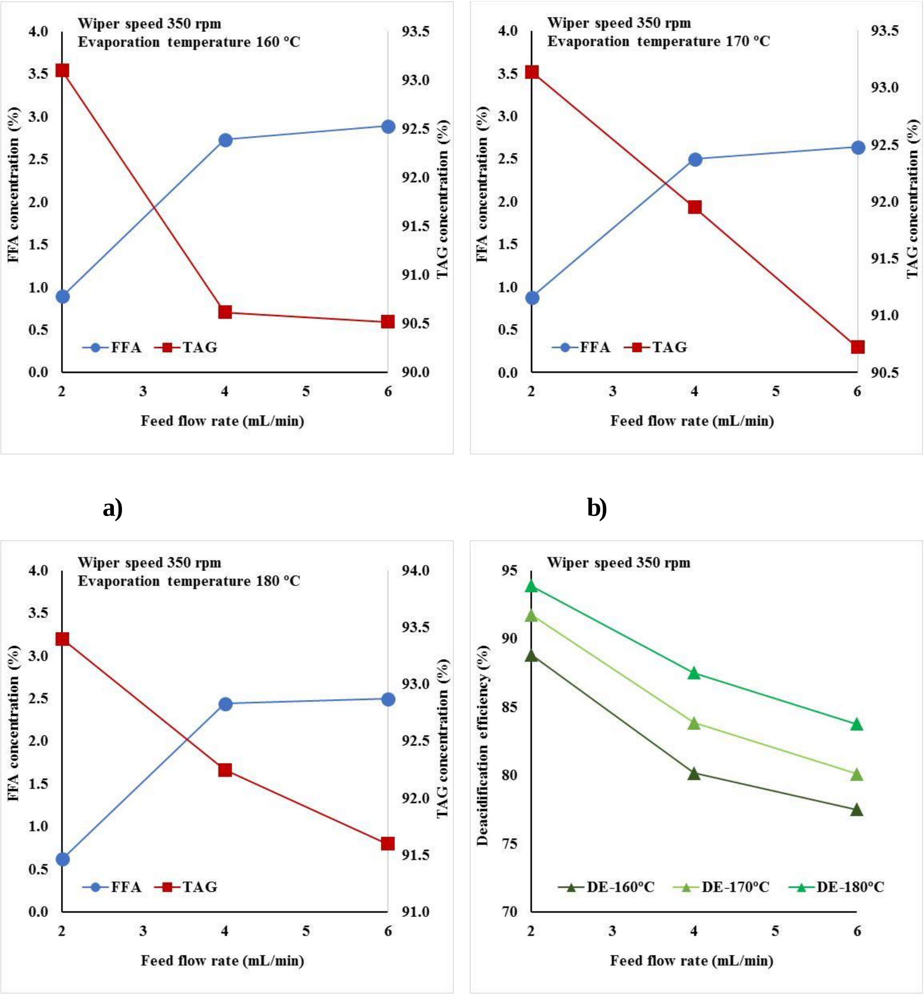
c)

Fig. 10. Effect of wiper rolling speed on: a) FFA and TAG composition in deacified oil at 160ºC, b) FFA and TAG composition in deacified oil at 170ºC c) deacidification efficiency

Feed flow rate

A decrease in TAG concentration and an increase in FFA concentration were noticed with increasing feed rate from 2 mL/min to 6 mL/min, for all evaporation temperatures studied (160ºC-180ºC). At low wiper speed (250 rpm) the FFA content does not vary too much, but with increasing wiper speed (350 rpm), their concentration varies significantly. This is because the feed flow influences the heat transfer in the separation system. If the feed flow rate increases, more energy will be needed to evaporate the components in the mixture. Fig.11 shows the influence of the feed flow rate at a constant wiper speed of 350 rpm for the three evaporation temperatures studied. The FFA concentration increased with two percent by increasing the feed rate from 2 mL/min to 6 mL/min. Thus, an increase was noticed from 0.89% to 2.89% in the case of distillation at 160ºC, from 0.88% to 2.64% for distillation at 170ºC, respectively from 0.62% to 2.50% at 180ºC. The TAG content decreased by increasing the flow with 2.77% at 160ºC, 2.60% at 170ºC and with 1.93% at 180ºC. The deacidification efficiency (fig.11d) decreased for all evaporation temperatures studied with 12.73% at 160ºC, 12.68% at 170ºC, respectively with 10.78% at 180ºC by increasing the feed rate from 2 mL/min at 6 mL/min.

Thus, experimental studies of camelina oil deacidification by molecular distillation showed an efficiency of over 90% and a FFA concentration in the heavy product below 1%. Three of these parameters (evaporation temperature, wiper speed and feed rate) influence the deacidification efficiency with 36%. The best results were obtained at 200ºC, 300 rpm and 2 mL/min, when 90.5% of the FFA was removed and the TAG concentration increased by ≈10% in the refined oil compared to the crude oil.



a) b)

c) d)

Fig. 11. Effect of feed flow rate at constant wiper speed (350 rpm) on a) FFA and TAG concentration in deacified oil at 160 ºC, b) FFA and TAG concentration in in deacified oil at 170 ºC, c) FFA and TAG concentration in in deacified oil at 180 ºC, d) deacidification efficiency

3.3.Deacidification process optimization

The objective of the study was to identify the optimal conditions for the separation of FFA from *Camelina sativa* oil by molecular distillation, using the response surface method. Based on statistical techniques, a second-order polynomial model was formulated to describe the system, effects and interactions between factors on the three answers identified in the experimental step (eq.1).

$$Y_i = \beta_{0,i} + \beta_{1,i}X_1 + \beta_{2,i}X_2 + \beta_{3,i}X_3 + \beta_{4,i}X_1X_2 + \beta_{5,i}X_2X_3 + \beta_{6,i}X_1X_3 + \beta_{7,i}X_1^2 + \beta_{8,i}X_2^2 + \beta_{9,i}X_3^2 \qquad (1)$$

Where Y_i represents the response variable of the optimization model, X_1, X_2, X_3 represent the three factors that influence the answers, $\beta_{(0,i)}$ is the regression coefficient with constant value, as the value of the response when there is no effect, $\beta_{(j,i)}$ represent the regression coefficients for the effects of factors X_i. For the deacidification process, three responses and three factors were identified, as shown in Table 3. To avoid extreme conditions, evaporation temperatures between 160-180ºC, wiper speeds between 250-350 rpm and feed rates between 2-6 mL/min were chosen for optimization.

Tabel 3. Independent variables for design (coded and encoded).

Independent variables (effect factors)			**Minim value**	**Central value**	**Maxim value**
Evaporator temperature (ºC)	Uncoded	X_1	160	170	180
	Coded		-1	0	1
Wiper rolling speed (rpm)	Uncoded	X_2	250	300	350
	Coded		-1	0	1
Feed flow rate (mL/min)	Uncoded	X_3	2	4	6
	Coded		-1	0	1

Three dependent variables (responses) were explored:

- the FFA concentration in heavy product - Y_1
- the deacidification efficiency - Y_2
- the TAG composition in heavy product -Y_3

For each answer a second-order polynomial model (eq.1) was proposed, considering the effect of the three independent variables (X_1, X_2, X_3), the interaction between them (X_1X_2, X_1X_3, X_2X_3) as well as the quadratic effect (X_1^2, X_2^2, X_3^2). The experimental matrix (Box-Behnken-BBD three-factor experimental model) consists of 17 experiments, of which 5 experiments represent replicates at the central points to establish the pure error (Table 4)

Tabel 4. BBD matrix with three factors on three levels and five replications in the central point

Experimental run	Variable		
	X_1	X_2	X_3
1	-1	-1	0
2	+1	-1	0
3	-1	+1	0
4	+1	+1	0
5	-1	0	-1
6	+1	0	-1
7	-1	0	+1
8	+1	0	+1
9	0	-1	-1
10	0	-1	+1
11	0	+1	-1
12	0	+1	+1

13	0	0	0
14	0	0	0
15	0	0	0
16	0	0	0
17	0	0	0

The values of the regression coefficients were determined and the p-value parameter with 95% confidence interval was calculated to determine the statistical significance of the regression models. The regression model is significant at 5% level if the *p*-value is smaller than 0.05. It was noticed that certain terms do not have a significant effect on the answers Y_1, Y_2 and Y_3 (p-value> 0.05). The FFA concentration in the heavy product is not influenced by the quadratic term X_2 or by the interaction between X_1X_3. The deacidification efficiency is not influenced by the quadratic term X_3, and the TAG concentration in the heavy product by the quadratic term X_1 and the interaction between X_1X_2. Equations 2-4 represent the second order polynomial models for evaluating the effect of the three factors on the responses of camelina oil deacidification by molecular distillaton.

$$Y_1 = 2.510 - 0.270X_1 - 0.296X_2 + 0.499X_3 + 0.155X_1X_2 + 0.363X_2X_3 + 0.020X_1X_3 + 0.201X_1^2 + 0.039X_2^2 - 0.361X_3^2 \quad (2)$$

$$Y_2 = 75.410 + 5.570X_1 + 8.939X_2 - 3.966X_3 - 1.903X_1X_2 - 2.015X_2X_3 + 2.328X_1X_3 - 2.298X_1^2 + 1.735X_2^2 - 0.090X_3^2 \quad (3)$$

$$Y_3 = 91.412 + 0.671X_1 + 0.401X_2 - 0.963X_3 - 0.010X_1X_2 - 0.353X_2X_3 + 0.157X_1X_3 + 0.074X_1^2 - 0.381X_2^2 + 0.426X_3^2 \quad (4)$$

Table 5 shows the values of the three responses calculated with the regression models, compared to the experimental data. A close correlation was found between the two data sets. The response surfaces obtained with the regression models are shown in Fig. 12-14

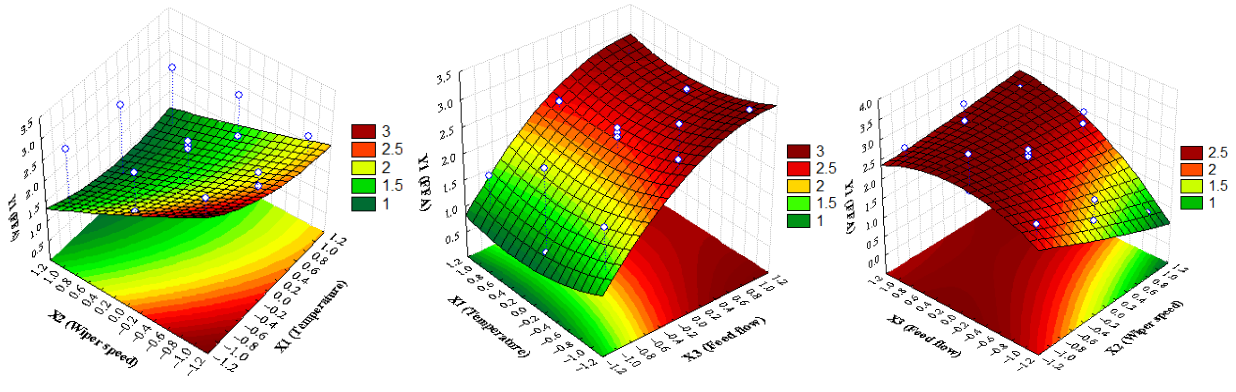
On these surfaces a point or a region/regions can identify where the answers have the optimal values (minimum or maximum). The response surfaces were performed in STATISTICA© software, with the intervals of the factors varying between the minimum and maximum values of the answers predicted by the quadratic model. From the surfaces of Y_1 (Fig.12a,b,c) it was noticed that in the regions of higher X_1 and X_2 and lower X_3, the concentration of FFA is minimal. Under these conditions, a region is identified in which the concentration of FFA in the heavy product is minimal (≈1%) for $-0.2<X_1<0.2$, $0.6<X_2<1.2$ and $-1.2<X_3<-0.8$.

Tabel 5. Experimental vs predicted data with second-order model.

Exp.	X_1 (°C)	X_2 (rpm)	X_3 (mL/min)	Y_1 (% FFA)		Y_2 (% DE)		Y_3(% TAG)	
				Exp.	Predicted	Exp.	Predicted	Exp.	Predicted
1	160	250	4	3.37	3.47	58.37	58.44	89.94	90.02
2	180	250	4	2.46	2.62	73.33	73.38	91.61	91.39
3	160	350	4	2.73	2.57	80.17	80.12	90.62	90.85
4	180	350	4	2.44	2.34	87.52	87.45	92.25	92.17
5	160	300	2	2.13	2.14	73.92	73.75	92.62	92.36
6	180	300	2	1.61	1.56	80.39	80.23	93.34	93.39
7	160	300	6	3.05	3.10	61.00	61.16	90.17	90.12
8	180	300	6	2.61	2.60	76.78	76.95	91.52	91.78
9	170	250	2	2.46	2.35	69.96	70.07	91.49	91.67
10	170	350	2	0.88	1.03	91.75	91.98	93.14	93.17
11	170	250	6	2.77	2.62	66.39	66.17	90.48	90.45
12	170	350	6	2.64	2.75	80.12	80.01	90.72	90.54
13	170	300	4	2.51	2.51	75.34	75.41	91.34	91.41
14	170	300	4	2.45	2.51	75.32	75.41	91.46	91.41
15	170	300	4	2.53	2.51	75.35	75.41	91.60	91.41
16	170	300	4	2.44	2.51	75.34	75.41	91.35	91.41
17	170	300	4	2.62	2.51	75.33	75.41	91.31	91.41

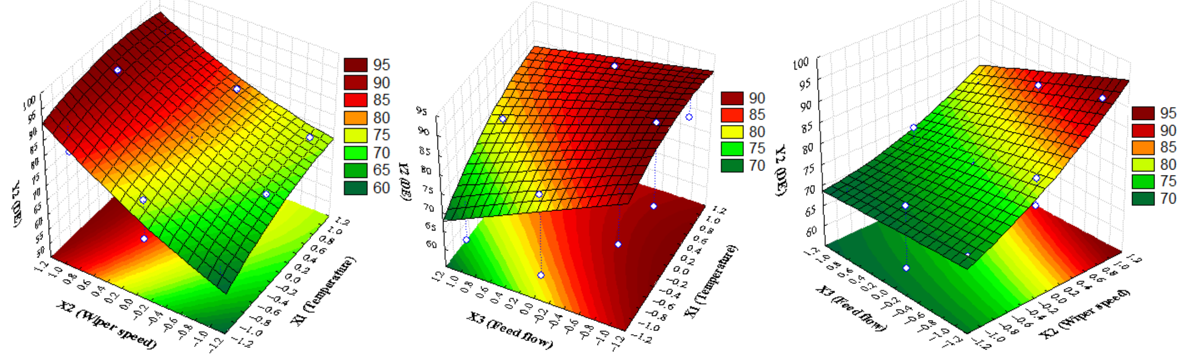
The surfaces of the Y_2 response (deacidification efficiency) show regions of maximum and minimum values (Fig.13a,b,c). Regions for maximum efficiency are obtained for high X_1 and X_2 and low X_3 conditions. The following regions were identified with values of deacidification efficiency greater than 95% for $0<X_1<1.2$, $0.6<X_2<1.2$ and $-1.2<X3<-0.8$. From the response surfaces for model Y_3 it was noticed that maximum concentrations of TAG over 93.5% can be obtained in the deacidified oil under the same conditions of high X_1 and X_2 and low X_3 (Fig.14a,b,c). The variation domains of the factors are: $0<X_1<1.2$, $0.6<X_2<1.2$ and $-1.2<X3<-0.8$.

In order to identify the optimal values of the responses, 2D graphs were computed in which each factor varies between -1 and +1 and the others have a constant value (identified from the response surfaces). In Figure 15a, three profiles of response Y_1 affected by X_1, X_2 and X_3 are presented, these profiles showing minimum values. Profiles presented in Figure 15b-c represent the responses of Y_2 and Y_3 affected by the same factors. These profiles showed maximum values. All three response profiles presented the optimal values at $X_1 = 0.34629$, $X_2 = 0,99996$, and $X_3 = -1$. These values were in the regions identified from the response surfaces.



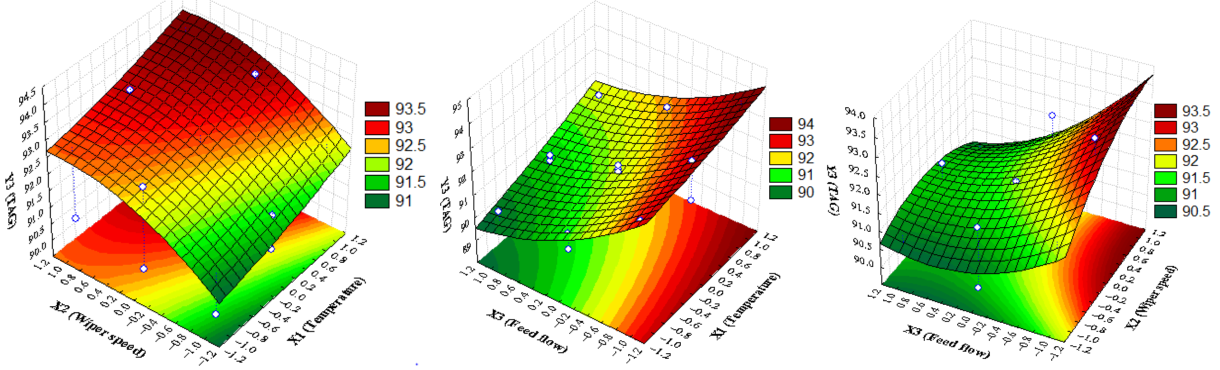
a) **b)** **c)**

Fig. 12. Response surface for Y_1 base on: a) X_1X_2 effects, X_3=ct, b) X_1X_3 effects, X_2=ct, c) X_2X_3 effects, X_1=ct



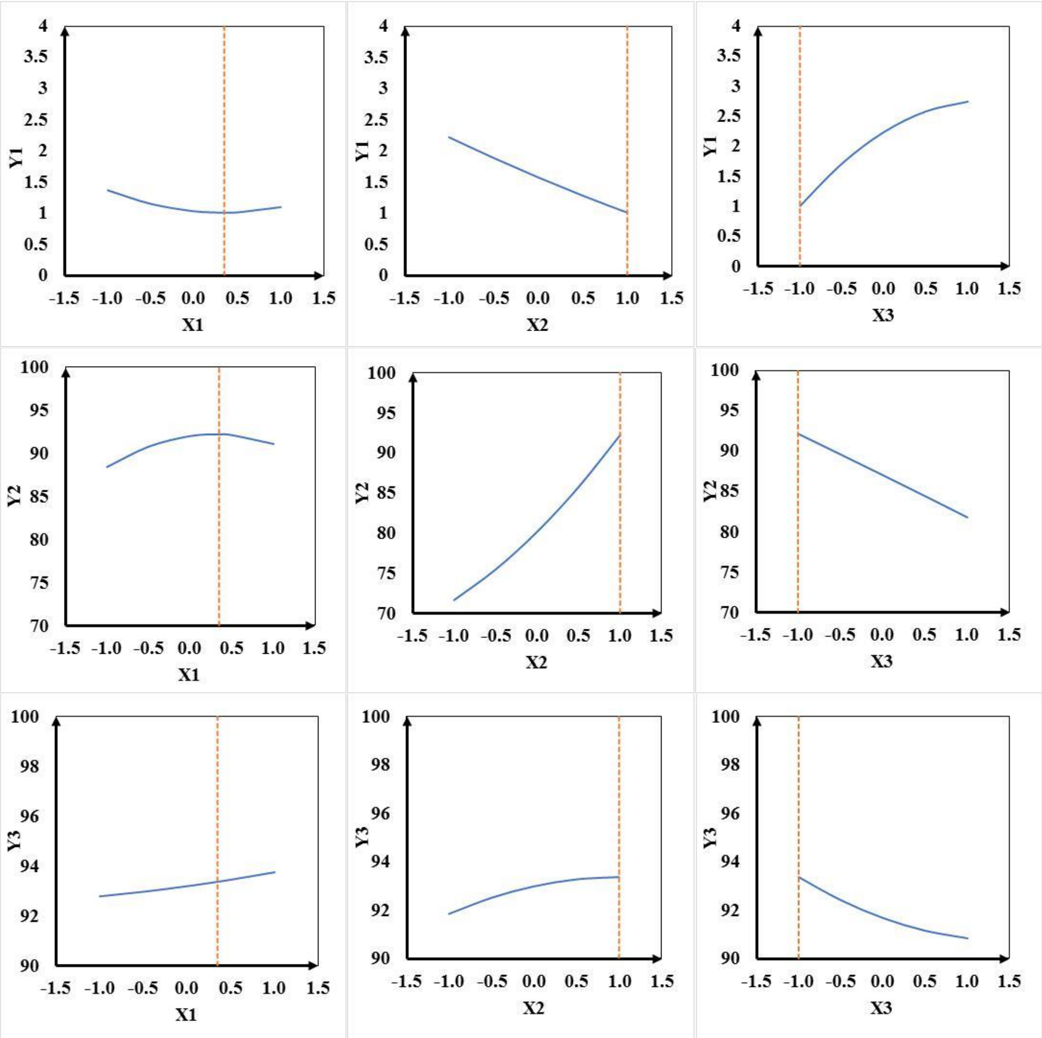
a) **b)** **c)**

Fig. 13. Response surface for Y_2 base on: a) X_1X_2 effects, X_3=ct, b) X_1X_3 effects, X_2=ct, c) X_2X_3 effects, X_1=ct



a) **b)** **c)**

Fig. 14. Response surface for Y_3 base on: a) X_1X_2 effects, X_3=ct, b) X_1X_3 effects, X_2=ct, c) X_2X_3 effects, X_1=ct



a) b) c)

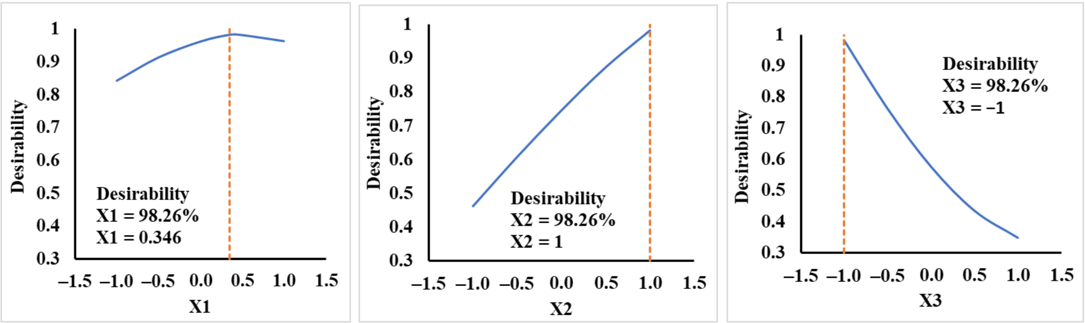
Fig. 15. Optimal responses: a) X1 effects, b) X2 effects, c) efect X3 effects

The individual desirabilities of the three responses were determined using the desirability functions proposed by Wu [107]. This function takes values between 0 and 1, where 0 was for an undesirable response (response is greater than a target value for minimum point or less than the smallest value for maximum) and 1 indicated a completely desirable response (response is less than the smallest value for minimum point or greater than a target value for maximum point).

Tabel 6. Desirability function parameters

Optimization objective	Response	Smallest value / Desirability	Medium value / Desirability	Target value / Desirability
Minimization	Y_1 (FFA)	0.88	2.12	3.37
		1	0.5	1
Maximization	Y_2 (DE)	58.37	75.06	91.75
		0	0.5	1
Maximization	Y_3 (TAG)	90.04	91.70	93.36
		0	0.5	1

Intermediate values of responses between smallest value and target value represented desirable responses. Smallest, medium, and target values for desirability functions are presented in Table 6, considering a linear variation for intermediate values. The profiles obtained by computing the the individual desirability functions with STATISTICA© for factor values between −1 and 1 are presented in fig.16.



a) **b)** **c)**

Fig. 16. Optimal desitability for: a) X_1 factor, b) X_2 factor, c) X_3 factor

In Table 7, the optimal values of responses and factors are shown. Final desirability function, defined as geometric mean of individual desirability functions, was 0.9826 for all factors X_1, X_2, X_3 combined.

Tabel 7. Optimal factors, optimal responses, and desirability

Optimization objective	Optimal response	Optimal factors	Individual desirability	Desirability
Minimization	Y_1 (%FFA) = 1.007	X_1= 0.34629	0.9488	0.9826
		X_2= 0.99996	1.0000	
		X_3= -1	1.0000	
Maximization	Y_2 (%DE) = 92.167	X_1= 0.34629	0.9488	0.9826
		X_2= 0.99996	1.0000	
		X_3= -1	1.0000	
Maximization	Y_2 (%TAG) = 93.360	X_1= 0.34629	0.9488	0.9826
		X_2= 0.99996	1.0000	
		X_3= -1	1.0000	

These optimal values of coded factors correspond to optimal operating conditions such as: evaporator temperature of 173.5 °C, wiper rolling speed of 350 rpm, and a feed flow rate of 2 mL/min for the deacidification process of *Camelina sativa* oil by molecular distillation. At these conditions, separation parameters for this process were minimum composition of FFA in bottom product of 1.007% FFA, maximum deacidification efficiency of 92.167%, and maximum concentration of TAG in bottom product of 93.360% TAG, with a desirability of 0.9826.

CHAPTER 4

ω-PUFA METHYL ESTERS CONCENTRATION SCHEMES BY MODERN SEPARATION METHODS. OBTAINING VALUE-ADDED COMPOUNDS.

In this chapter the separation of ω-PUFA fatty acid methyl esters (C18:2, C18:3) from camelina oil was studied. ω-PUFA are valuable compounds used in the food supplements industry, but they are found in mixtures with saturated and unsaturated fatty acids and separation by traditional methods is difficult to achieve. In order to increase the volatility of ω-PUFA, a preliminary transesterification step of camelina oil with methanol was performed, obtaining ω-PUFA methyl esters. Four strategies were studied based on separation by molecular distillation and high vacuum distillation (fig.1-4). The first three schemes are focused on molecular distillation in different distillation stages of the light or heavy product using a pilot laboratory-scale molecular distillation equipment KDL5 model from UIC GmbH. The last strategy consisted of two high vacuum distillation stages using a pilot laboratory scale distillation equipment DSL5 model from UIC GmbH where in the first stage the light component was removed and in the second stage the heavy components (fig. 4).

In molecular distillation the separation is achieved by shifting the evaporated molecules and not based on differences in volatility as in the case of conventional distillation, thus allowing the separation of compounds with similar boiling points but with different volumes. This technique is characterized by several important aspects: short residence time of compounds in the area exposed to heat, low operating temperature due to the vacuum created in the evaporation space and a characteristic mechanism of mass transfer in the space between evaporation and condensation surfaces. The process involves compounds vaporization and the movement of the vaporized molecules over a short distance of only a few centimeters, from the evaporation surface to the condensation surface. The distance travelled by a molecule between two successive collisions is called the mean free path. Under high vacuum conditions (0.1 Pa), the distance between the evaporator and the condenser is less than or equal to the mean free path.

The scientific research consisted of experimental determinations on:

- obtaining the raw materials (FAME mixtures) starting from an oil with a high content of polyunsaturated fatty acids (*Camelina sativa* oil);
- analysis of raw materials by qualitative and quantitative methods to determine the ω-PUFA esters content;

- development of four separation schemes by molecular distillation and high vacuum distillation for the concentration of ω-PUFA methyl esters;
- analysis of separation products to determine their purity;

4.1. Raw material

The oil was subjected to a transesterification reaction with methanol and KOH, in ten experiments, resulting in 4000 g of FAME which was divided into four batches of 1000 g each. The transformation yield was between 95.6-96.6% and the reaction product is a mixture of saturated (SFA), unsaturated (MUFA), ω-PUFA methyl esters and unreacted glycerides. Table 8 shows the compositions of the four batches according to the GC-MS analysis.

Tabel 8. FAME composition for the four batches

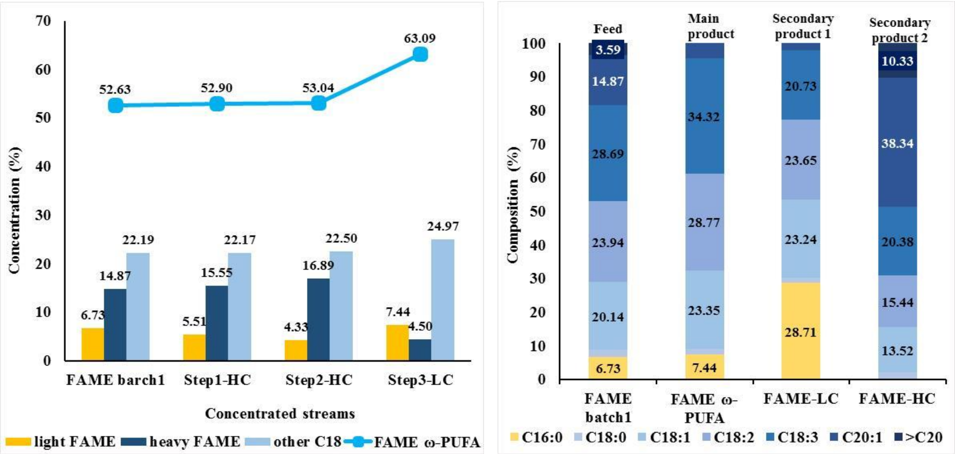
Nr.	Component	Composition			
		Batch 1±SD (%)	**Batch 2±SD (%)**	**Batch 3±SD (%)**	**Batch 4±SD (%)**
1	<C16	-	-	-	0.18±0.02
2	C16:0	6.73± 0.16	6.92 ± 0.11	6.84 ± 0.10	7.46±0.10
3	C18:0	2.05 ± 0.08	2.21 ± 0.05	1.97 ± 0.07	2.03±0.03
4	C18:1	20.14 ± 0.17	20.41 ± 0.23	20.22 ± 0.15	18.70±0.20
5	C18:2 (ω-6)	23.94 ± 0.29	23.48 ± 0.09	23.97 ± 0.25	18.19±0.10
6	C18:3 (ω-3)	28.69 ± 0.39	27.75 ± 0.33	29.15 ± 0.58	34.59±0.19
7	C20:1	14.87 ± 0.45	15.59 ± 0.14	15.21 ± 0.21	16.46±0.13
8	>C20	3.58 ± 0.58	3.64 ± 0.12	2.64 ± 0.42	2.38±0.35
9	ω-PUFA	52.63	51.23	53.12	52.78

4.2. Experimental models for ω-PUFA methyl esters separation

For the concentration of ω–PUFA methyl esters obtained from camelina oil, four separation schemes are proposed considering different molecular distillation steps for the separation of light or heavy volatile components. The optimal operating conditions were established based on the properties calculated in the previous chapter but also based on experimental trials.

4.2.1. Three steps model molecular distillation strategy (TSM-MD)

According to this MD model, ω-polyunsaturated fatty acid methyl esters (PUFA-ME) were concentrated in the heavy distillation product, which was further used in two other consecutive distillation steps. The strategy adopted was to separate the light esters in distillate in the first two steps and ω-PUFA methyl esters in distillate in the third step. Three products are obtained: main product (concentrated ω-PUFA methyl esters), secondary product 1 (light component FAME-LC) and secondary product 2 (heavy component FAME-HC). Figure 17a shows the variation of ω-PUFA, light and heavy methyl esters concentration according to the number of molecular distillation steps. For steps 1 and 2 the separation occurred at 56ºC and 0.1Pa, the light molecules of C16:0 condensing and those of ω-PUFA methyl esters remaining in the heavy stream.



a) b)

Fig. 17. TSM-MD methyl esters compositions: a) of light, heavy and ω-PUFA methyl esters in concentrated streams, b) of FAME in final products

It can be noticed that in Step1-HC stream, the concentration of C16:0 decreases from 6.73% to 5.51% while in the distillate stream C16:0 is concentrated until 30%. ω-PUFA methyl esters concentration remained almost constant (increasing from 52.63% in the raw material to 52.90% in Step1-HC, and to 53.04% in Step2-HC). In the third step, when the ω-PUFA methyl esters were collected in the light product (evaporation temperature was setup at 85^{0}C and 0.1Pa), their concentration increased to 63.09 %. In Fig.17b the compositions of all separation products are presented, reported on FAME initial compositions (feed). The main product contains 90% esters with 18 atoms of carbon. Concerning the other products, their composition is different: FAME-LC product contain 28.71% of the most volatile compound (C16:0), while the FAME-HC is rich in heavy compounds (C20:1 composition is almost 38%). Due to the operation conditions chosen for this model, FAME-LC product does not content the heaviest compounds, while the lighter compound was removed from the FAME-HC stream. These secondary products can be used as biodiesel component.

In Fig.18, total mass balance for each step is presented. After one MD step, the degree of separation (defined as ratio of amount collected as light product and feed) is 5.66%, for second step is 5.80% and for third step is 38.10%. Finally, after three MD steps, 53.08% concentrated ω-PUFA ME was obtained from 1000 g raw material.

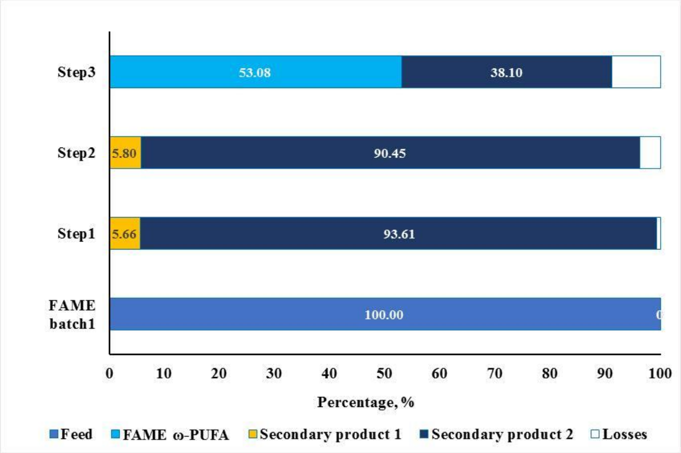
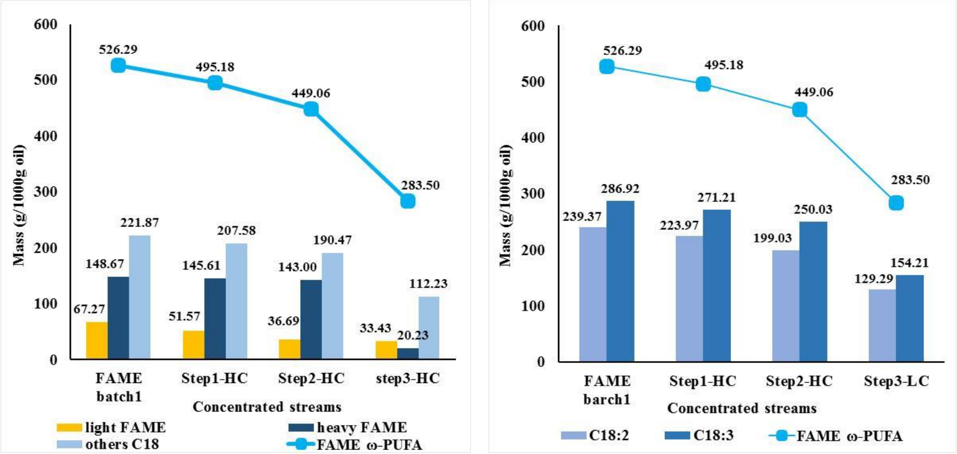


Fig. 18. Total mass balance for TSM-MD separation strategy

Fig.19a shows the mass balance of ω-PUFA ME for each molecular distillation step. It started from a raw material with 526.29 g ω-PUFA ME content and after the first molecular distillation step 24.71 g passed into the light product and 495.18 g into the heavy product (1-HC step). After the second MD step, 24.48 g passed into the light product and 449.06 g into the heavy product (2-HC stage), and after the last stage 285.50 g passed into the light product (3-LC stage) and 115.55 g in the heavy product. In addition to the content of ω-PUFA ME, the content of light FAME (C16:0) and heavy FAME (C20:1, C22:1) of the main distillation streams is also presented. Fig.19b shows the distribution of ω-3 PUFA and ω-6 PUFA ME from the distillation streams.



a) **b)**

Fig. 19. Mass balance for TSM-MD strategy: a) light/heavy FAME and ω-PUFA ME in the final distillation streams, b) C18:2, C18:3 ME in the final distillation streams

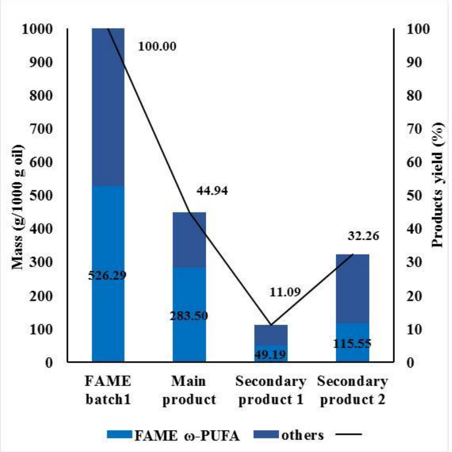


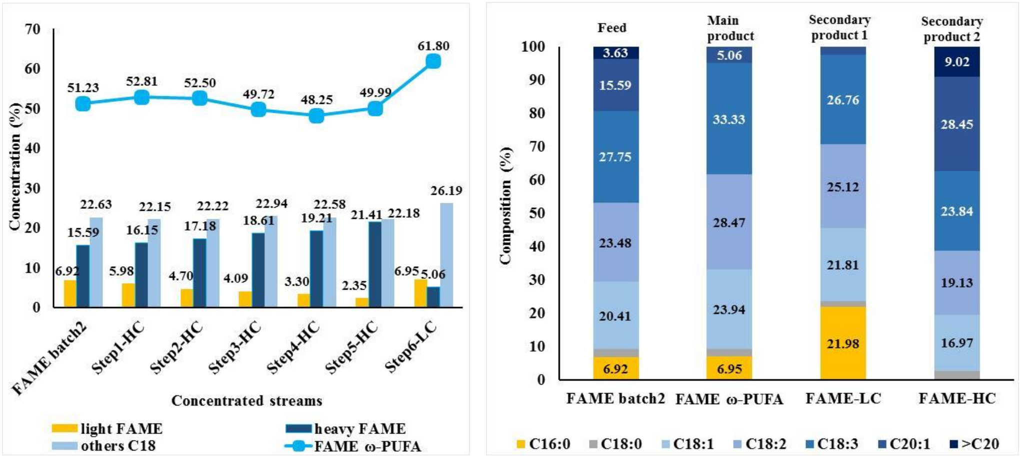
Fig. 20. Mass balance and yield of final distillation products for TSM-MD strategy

Fig.20 shows the ω-PUFA ME mass from the raw material, main product concentrated in ω-PUFA ME (step 3-LC), secondary product 1 (step 1-LC+step -LC) and secondary product 2 (step 3-HC) compared to the other components found in the composition of the respective streams. The yield of the main distillation products is also presented. 1000 g of raw material was used from which 449.39 g of concentrated product in methyl esters ω-PUFA were obtained, with a yield of 44.94%, 110.88 g of by-product 1, with a yield of 11.09% and 322.55 g by-product 2, with a yield of 32.26%.

4.2.2. Six steps model molecular distillation strategy (SSM-MD)

Previous experiment showed that it is difficult to totally remove the most volatile component (C16:0) from the mixture in two steps. It has been investigated if increasing the number of distillation steps leads to removal of FAME and consequently ω-PUFA ME concentration.

The strategy proposes the removal of C16:0 in five molecular distillation stages and the concentration of ω-PUFA ME in the light product from the sixth stage. Using SSM-MD strategy the following products were obtained: main product (concentrated ω-PUFA ME), one secondary light product (light FAME from steps1-5, named secondary product 1) and one heavy product (heavy FAME from sixth step, named secondary product 2).



a) **b)**

Fig. 21. SSM-MD methyl esters compositions: a) of light, heavy and ω-PUFA methyl esters in concentrated streams, b) of FAME in final products

Fig 21a shows the variation of FAME concentration in the concentrated streams. After five MD steps, light esters are removed until 2.35%, each step decreasing by 13% to 28% against feed composition. This modification implies the increasing of heavy esters concentration from 15.59% to 28.45%, while the composition of esters with 18 atoms of C remaining almost constant. So, increasing number of MD steps, do not concentrate ω-PUFA ME higher than TSM-MD strategy. At the last step, by increasing the evaporating temperature, ω-PUFA ME and light esters migrate to the distillate stream (61.8% concentration) while in the bottom stream heavy FAME is collected. C18:0 and C18:1 remained almost constant in the first five steps. In the last step, when the ω-PUFA methyl esters concentration was performed in the light product, the concentration of C16:0 increased slowly, but the concentration of C20:1 decreased so that the concentration of ω-PUFA methyl esters turned out to be higher. C18:0 was constant and C18:1 increased slightly. The compositions of final products are presented in Fig.21b. Light streams from steps 1 to 5 are collected as a single product with ≈22% C16 ester. Main product contains small amounts of heavy FAME (5% C20:1) and has the same concentration of C16:0 as the feed. Secondary product 2 represents the heavy product from sixth MD step and contains approximately 37% C20-C22. It does not contain any light components C16:0.

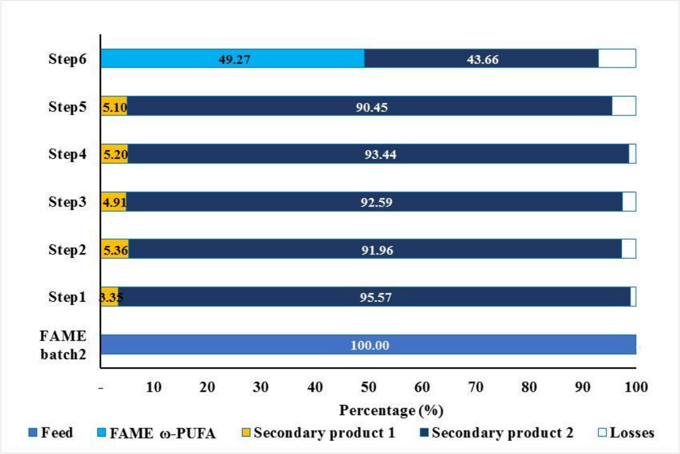
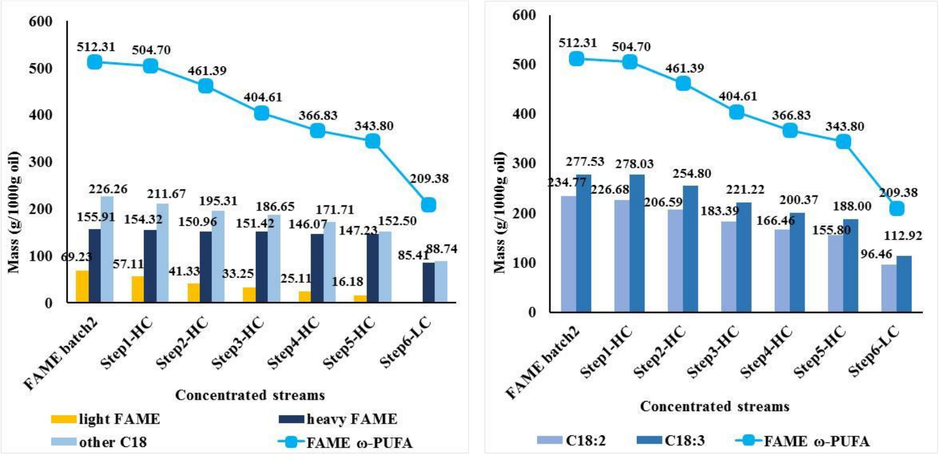


Fig. 22. Total mass balance for TSM-MD separation strategy

Regarding the mass balance, the results are comparable to those of the three-stage model (fig.22). Light products represent between 4-5% in each distillation step. The heavy product collected in step 5 represented approximately 688 g and was separated in the last step as 49.27% light product (ω-PUFA ME concentrate, main product) and 43.66% heavy product (secondary product 2).



a) b)

Fig. 23. Mass balance for SSM-MD strategy: a) light/heavy FAME and ω-PUFA ME in the final distillation streams, b) C18:2, C18:3 ME in the final distillation streams

The mass of ω-PUFA ME gradually decreased with about 10% in each distillation step (fig. 23a). After the last step, 209.38 g of ω-PUFA ME with a concentration of 61.80% were concentrated in the light stream. This amount is 25% lower compared with the one obtained using the TSM-MD strategy, while the concentration is 2% lower. Fig.23b shows the distribution of ω-3 and ω-6 ME from the raw material and concentrated streams. Fig.24 shows the mass of ω-PUFA ME in the raw material, the main product concentrated in ω-PUFA ME (step 6-LC), the secondary product 1 (step 1-LC + step 2-LC +… + step5-LC) and secondary product 2 (step 6-HC) compared to the other components found in their composition.

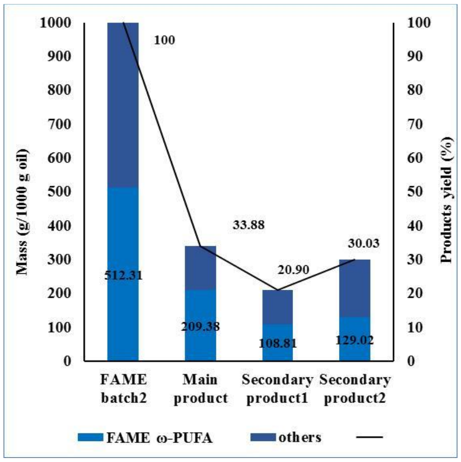
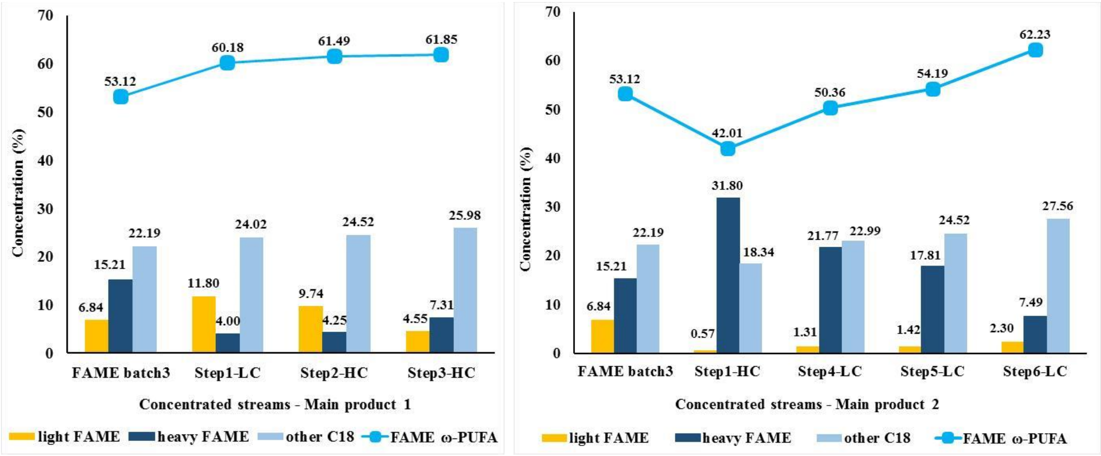


Fig. 24. Mass balance and yield of final distillation products for SSM-MD strategy

The figure also shows the yield of the main distillation products. 1000 g of raw material were used (batch 2) from which 338.81 g of the main product were obtained with a yield of 33.88% containing 209.38 g of ω-PUFA methyl esters and 129.43 g of other components, 209 g of secondary product 1 with a yield of 20.9% containing 108.81 g of ω-PUFA methyl esters and 100.2 g of other components and 300 g of secondary product 2 with a yield of 30.03%, containing 129 g of ω-PUFA methyl esters and 171.24 g of other components.

4.2.3. Combined steps model molecular distillation strategy (CSM-MD)

This model proposes the separation of the heavy components in the heavy distillation product from the first MD stage, the separation being performed at a higher temperature (88ºC). The light stream is concentrated into the next two steps and the heavy stream into the next three steps. Four products are obtained: main products 1 and 2 (concentrated ω-PUFA ME), secondary product 1 (light FAME) and secondary product 2 (heavy FAME). Fig.25a shows the composition of the main product 1 and fig.25b of the main product 2. To obtain the main product 1 the separation scheme followed the following route: feed-light product from step 1- heavy product from step 2- heavy product from step 3. The light components were concentrated in the light product fromstep 1 (11.80%) and their concentration droped to 4.55% in the last MD step. The ω-PUFA ME concentration significantly increased from the first MD step due to the high evaporation temperature, while in the following steps it increased slowly to a final concentration of 61.85%. A similar concentration was obtained in step 2 but the concentration of the light component was higher. Heavy FAME is found in low concentration due to the evaporation conditions. FAME components with 18 carbon atoms did not significantly change their composition during the separation scheme. The separation scheme for obtaining the main product 2 followed the following route: feed- heavy product from step 1- light product from step 4- light product from step 5-light product from step 6. The heavy product obtained in step 1 contains small amounts of light component (0.57% C16:0) and thus the ω-PUFA ME were concentrated in the light products in the following steps. After the next MD step the ω-PUFA ME concentration increased by 20% (50.36%), after two steps by 30% (54.19%) and after three steps by 48% (62.23%) compared to the ω-PUFA ME concentration from step 1.



a) **b)**

Fig. 25. FAME composition in the CSM-MD strategy: a) concentrated streams to obtain main product 1, b) concentrated streams to obtain main product 2

The quality of the final distillation products is presented in fig.26, compared to the initial composition of FAME. Both main products present an improved quality because they contain a lower concentration of light components (4.55-2.30%), a lower concentration of heavy components, a higher concentration of ω-PUFA ME (61.85-62.23%) and the same composition of methyl esters with 18 carbon atoms. Secondary product 1 contains 30% light component C16:0 and does not contains any heavy components, while the secondary product 2 does not contain light components but has approximately 71% C20 and > C20 methyl esters.

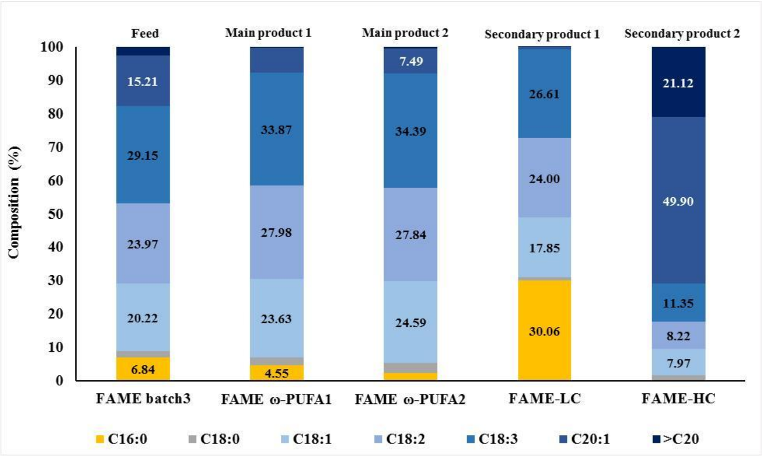


Fig. 26. The composition of the final distillation products obtained using SCM-MD strategy

The total mass balance for this model is presented in fig.27.

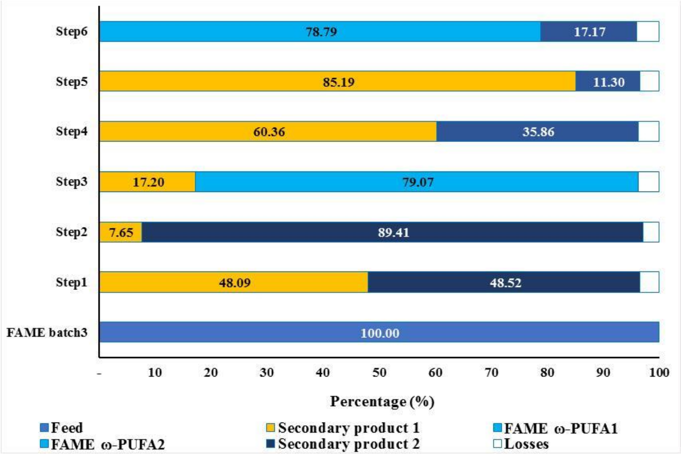
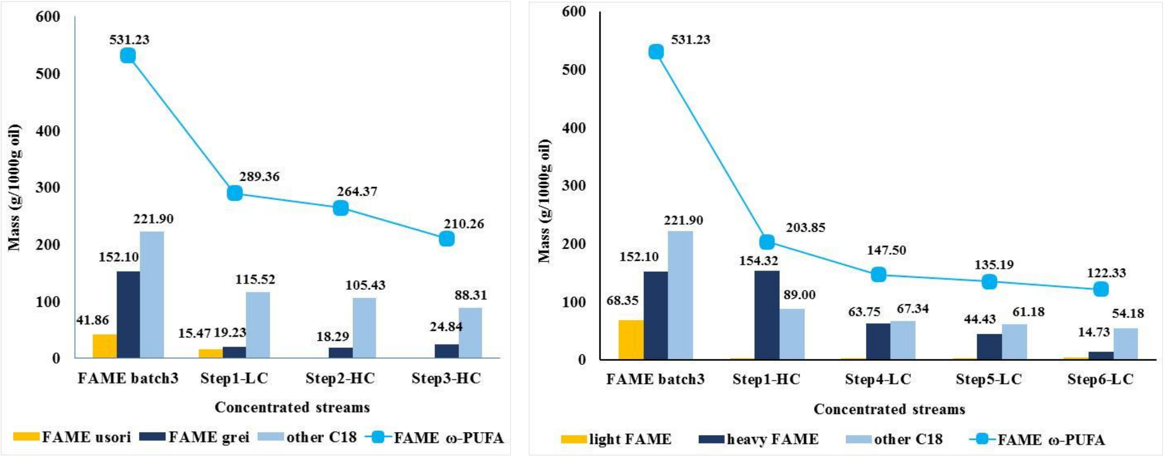


Fig. 27. Total mass balance for CSM-MD strategy

For each MD step, the degree of separation of light/heavy products is presented. In the first step, 48.09% of the methyl esters migrated into the light product and approximately the same amount into the heavy product. The light product obtained in step 1 was used in step 2 where 7.65% was separated as a light product while the heavy product was used in the next MD step. In step 3, 79.07% is separated as main product 1 (FAME ω-PUFA1) and 17.20% as light product. The heavy product obtained in step 1 (48.52%) was used in step 4 where 60.36% was concentrated as a light product. This product is further concentrated in step 5 where 85.19% light product is obtained and then in step 6 where 78.79% main product 2 (ω-PUFA2) is resulting.

Regarding the mass balance for ω-PUFA ME, 210.26 g were separated in the main product 1 and 122.3 g in the main product 2, representing a recovery of 62.60% of the initial amount of methyl esters ω-PUFA (fig.28)



a) **b)**

Fig. 28. Mass balance for CSM-MD strategy: a) concentrated streams to obtain main product 1, b) concentrated streams to obtain main product 2

Fig. 29 shows the degree of recovery for the final distillation products compared to the amount of FAME used as raw material, through these model 53.66% main products and 36.06% secondary products were obtained.

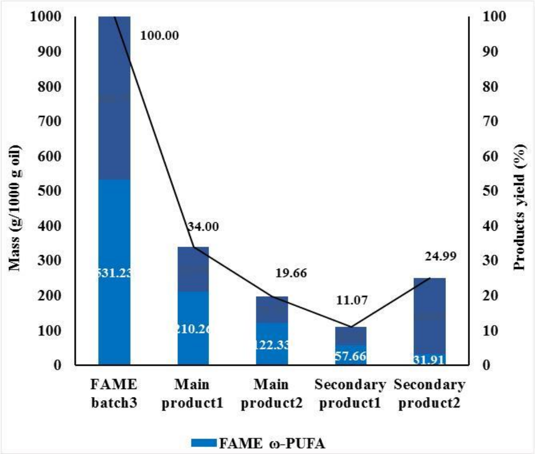
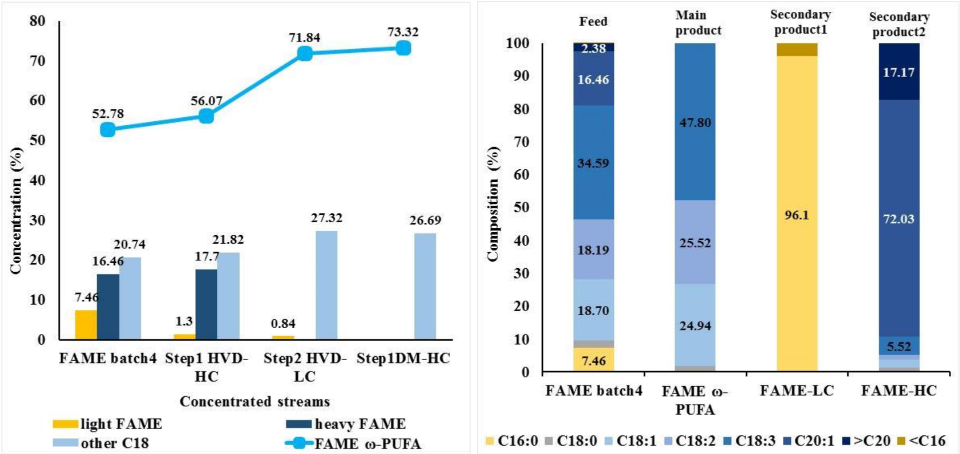


Fig. 29. Mass balance and yield of final distillation products for CSM-MD strategy

4.2.4. High vacuum distillation combined with molecular distillation strategy (HVD-MD)

According to this strategy, ω-PUFA ME were concentrated in the heavy distillation stream from the first HVD step, which was further used in the second HVD step and then purified in an additional MD step. The model aimed to concentrate the slightly volatile C16:0 methyl ester in the light product of the first HVD step, while in the heavy product, a mixture of ω-PUFA ME, C18 components and heavy esters can be found. This mixture was then separated in the second HVD step where a light stream highly concentrated in ω-PUFA ME and a heavy stream concentrated in C20 and >C20 components were obtained. The light product from second

HVD step was then purified in a MD step, ω-PUFA methyl ME being collected in the heavy product. Three products were thus obtained: a main product (ω-PUFA ME concentrate), second product 1 (FAME-LC light components concentrate) and second product 2 (FAME-HC heavy components concentrate).



a) **b)**

Fig. 30. HVD-MD methyl esters compositions: a) of light, heavy and ω-PUFA methyl esters in concentrated streams, b) of FAME in final products

Fig.30a shows the variation of ω-PUFA ME and light/heavy FAME concentration in the concentrated streams, according to the number of distillation steps. In the first HVD step the concentration of ω-PUFA ME increased by 5.86%, but in the second HVD step a concentration of about 22% was achieved due to the removal of the heavy components. By molecular distillation, the concentration of ω-PUFA ME slightly increased by 2% due to the removal of C16:0 traces and the slow decrease of the C18:1 and C18:0 methyl esters.

Fig.30b shows the compositions of the final separation products related to the FAME composition in the feed. The main product contains only esters with 18 carbon atoms of which 47.80% are ω-3 methyl esters (C18:3) and 25.52% ω-6 methyl esters (C18:2). The concentration of the C18:0 component remained constant while the one of C18:1 component increased by 28.70% compared to the raw material. Regarding the composition of the secondary products, FAME-LC (the light product obtained from HVD step 1) is a 96% concentrate of C16:0 ME. FAME-HC (heavy product from HVD step 2) is a 72% concentrate of C20:1 ME.

The total mass balance is presented in fig.31. For each distillation step, the degree of separation of light/heavy products is presented. In the first HVD step, more than 93% of the methyl esters migrated into the heavy product. This was further used in second HVD step where 71.80% of the methyl esters were colected into the light product which was used as a raw material for molecular distillation. 64.64% ω-PUFA ME concentrate was obtained.

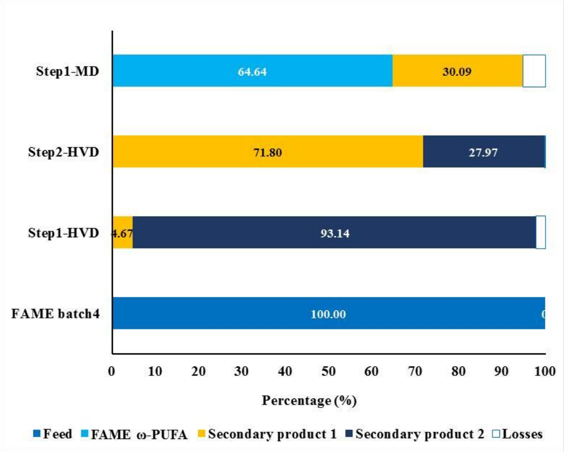
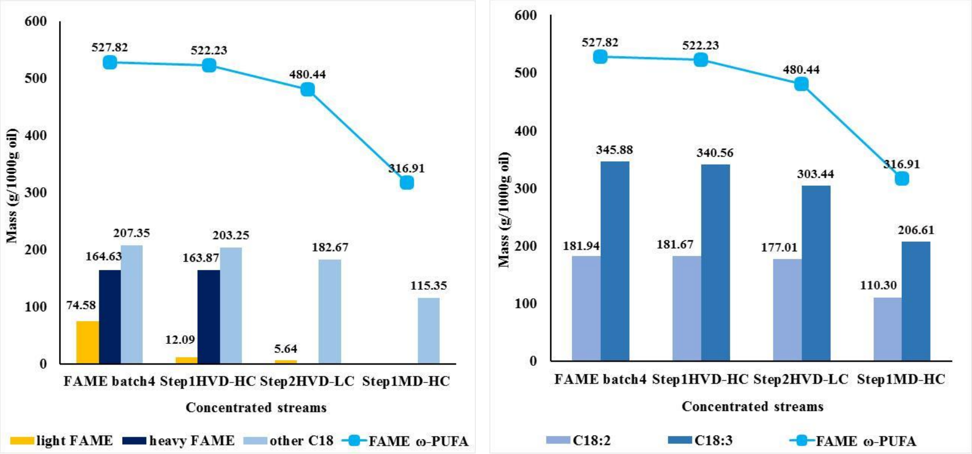


Fig. 31. Total mass balance for HVD-MD strategy

Regarding the mass balance for FAME components (fig.32), a raw material with 527.82 g ω-PUFA ME content was used and after the first HVD step 522.23 g passed into the heavy product (step 1 HVD-HC). After the second HVD step, 480.44 g ω-PUFA ME were collected in the light product (step 2 HVD-LC) from which 316.91 g ω-PUFA ME were finally obtained, with a recovery of 60% of the initial amount. Also, the amount of light components (light FAME - C16:0), heavy components (heavy FAME - C20:1) and other 18 carbon atoms components (C18:0, C18:1) from the main distillation streams is presented.

Fig.32b shows the distribution of ω-3 PUFA and ω-6 PUFA ME from the distillation products.



a) **b)**

Fig. 32. Mass balance for HVD-MD strategy: a) light/heavy FAME and ω-PUFA ME in the final distillation streams, b) C18:2, C18:3 ME in the final distillation streams

Fig.33 shows the mass of ω-PUFA ME in the raw material, the main product concentrated in ω-PUFA ME (stage 1 MD-HC), the secondary product 1 (stage 1 HVD–LC) and the secondary product 2 (stage 2 HVD - HC) compared to the other components found in the composition of these streams. The yield of the main distillation products is also presented. 1000 g raw material containing 527.82 g ω-PUFA ME was used, of which 432.26 g main product containing 316.91 ω-PUFA was obtained, 46.66 g of secondary product 1 containing only C16:0 and traces of components with less than 16 carbon atoms and 260.51 g secondary product 2 with 18.33 g of ω-PUFA methyl esters, the rest being heavy C20-C22 components.

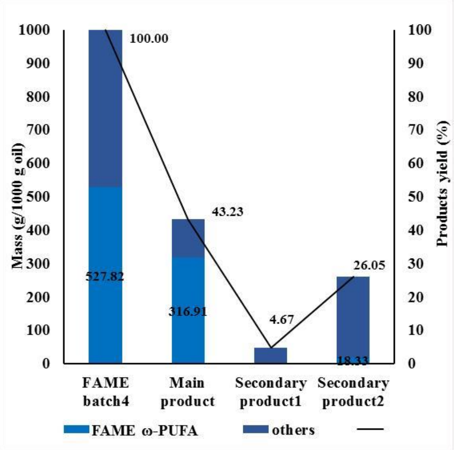


Fig. 33. Mass balance and yield of final distillation products for HVD-MD strategy

In terms of the ω-PUFA ME amount obtained in the main product, it was noticed that the CSM-MD strategy gave the best results, obtaining 536.52 g final product, of which 332.59 g are ω-PUFA ME. For this model, a separation yield of 62.61% was obtained and the concentration of ω -PUFA ME in the final product was about 62%. Regarding the purity of the main product, the HVD-MD strategy was the most efficient, obtaining a final product with 73.32% ω-PUFA ME and a separation yield of about 60%. In case of the TSM-MD strategy, a final product with a concentration over 63% ω-PUFA ME and a yield of approximately 54% was obtained. Increasing the number of distillation steps proved to be a disadvantage, obtaining the lowest separation efficiency (40.87%) and a concentration below 62% when SSM-MD strategy was used (Table 9).

Tabel 9. Experimental results for the proposed strategies

Strategy	ω-PUFA in the raw material (g)	ω-PUFA in the main product (g)	Yield (%)	ω-PUFA ME conc. in the final product (%)	Main product amount (g)	Other components in the main product (g)
TSM-MD	526.29	283.50	53.87	63.09	449.39	165.89
SSM-MD	512.31	209.38	40.87	61.80	338.81	129.43
CSM-MD	531.23	332.59	62.61	62.04	536.52	203.93
HVD-MD	527.82	316.91	60.04	73.32	432.26	115.35

Fig.34 compares the amount of ω-PUFA ME and their concentration in the main products obtained with the proposed strategies. It was noticed that the difference between the amount of ω-PUFA ME in the main products obtained in the CSM-MD and HVD-MD strategies is small (approximately 15 g), instead the concentration of ω-PUFA ME in the main product obtained by HVD-MD strategy is over 15% higher compared to the main product from CSM-MD strategy.

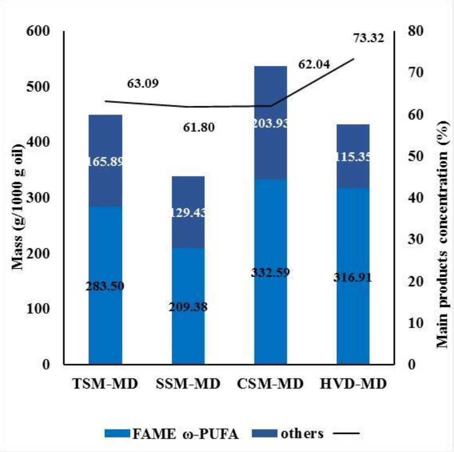
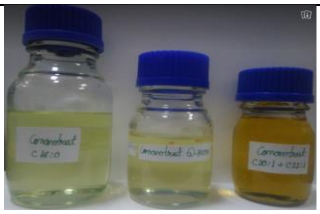
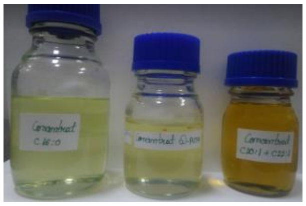
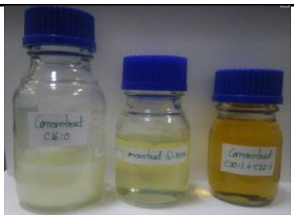


Fig. 34. Amount of ω-PUFA ME vs. concentration in the main products from the proposed strategies

4.2.5. Value-added products obtained

Separation strategy	Value-added product	
TSM-MD		*Main product:* 449.39 g/kg oil of 63.09% ω-PUFA ME concentrate
		Secondary product 1: 110.88 g/kg oil esters mixture with high content of methyl palmitate (28.71%)
		Secondary product 2: 322.55 g/kg oil esters mixture with high content of methyl eicosenoate and methyl docosenoate (48.67%)
		*Main product:* 338.38g /kg oil of 61.80% ω-PUFA ME concentrate
		Secondary product 1: 209.01 g/kg oil esters mixture with

SSM-MD		high content of methyl palmitate (21.98%)
		Secondary product 2: 300.26 g/kg oil esters mixture with high content of methyl eicosenoate and methyl docosenoate (37.47%)
CSM-MD		*Main product 1*: 339.95 g/kg oil of 61.85% ω-PUFA ME concentrate
		Main product 2: 196.56 g/kg oil of 62.23% ω-PUFA ME concentrate
		Secondary product 1: 110.75 g/kg oil esters mixture with high content of methyl palmitate (30.06%)
		Secondary product 2: 249.91 g/kg oil esters mixture with high content of methyl eicosenoate and methyl docosenoate (71.02%)
HVD-MD		*Main product 1*: 432.26 g/kg oil of 73.32 % ω-PUFA ME concentrate
		Secondary product 1: 46.66 g/kg oil esters mixture with high content of methyl palmitate (96.10%)
		Secondary product 2: 260.51 g/kg oil esters mixture with high content of methyl eicosenoate and methyl docosenoate (89.20%)

CHAPTER 5

CONCLUSIONS

The main objective of the PhD thesis is to obtain concentrates of ω-PUFA fatty acids esters (omega polyunsaturated fatty acid derivatives) starting from a vegetable raw material (*Camelina sativa* oil), using advanced separation methods. This resource is recommended for the low cost of the oil and the attractive profile of ω-PUFA fatty acids (≈ 52% ω-PUFA fatty acids). FAME mixtures obtained by transesterification reaction have a complex composition and their properties are very difficult to determine experimentally due to their thermolabile character. For the studied separation processes, under high vacuum conditions, properties such as the mixture boiling temperature, density and viscosity are very important and depend on the composition and system pressure. The vapor pressures for two alkyl esters (methyl linolete and ethyl oleate) were experimentally measured and different prediction models as correlations, group contribution and corresponding state principal methods were used to validate experimental data. The prediction method with the smallest error was further used to predict the vapor pressures for all the compounds founded in FAME mixture. Also, viscosities and densities of 16 FAME mixtures were experimentally measured and different prediction methods were used for validation.

The experimental research aimed to find a methodology for treating oils with different acidity (freshly pressed or with high acidity) by removing FFA that can influence the transesterification reaction by forming soaps. Conventional methods (alkaline treatment and washing with plenty of water) are disadvantageous due to high oil losses and large amount of generated wastewater. Experimental trials were performed to determine the effect of the operation parameters on the deacidification efficiency and using the response surface methodology the optimization of camelina oil deacidification by molecular distillation was made.

ω-PUFA ME obtained after transesterification of deacidified camelina oil with methanol (C18:2, C18:3) are difficult to separate because they have close boiling points and are thermosensitive components. At very low pressure (high vacuum conditions), the boiling temperatures drop below the degradation limit, but the differences between the esters boiling points are very small (1°C) and cannot be separated by classical distillation methods. Four strategies based on different steps of molecular distillation and high vacuum distillation, and different operation conditions were used in order to separare ω-PUFA ME.

The methods described in this paper can be applied to any type of oil, regardless of its acidity or composition. In case of deacidification process by molecular distillation the main advantage is that the oil losses are minimal and the wastewater treatment is eliminated. Using advanced separation methods for ω-PUFA ME concentration, as molecular distillation and high vacuum distillation, the problem of valuable compounds degradation is eliminated due to the low operating temperatures. Another advantage is that the processes did not require the use of chemical solvents. Besides the 73.32% ω-PUFA ME concentrate, two other value-added products were obtained: 96% methyl palmitate and 89.20% methyl eisosenoate/methyl dososenoate.

Publications and communications at scientific conferences on thesis subject

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Iancu, P., **Stefan, N.G.**, Plesu, V., Toma, A., Stepan, E., Advanced high vacuum techniques for ω-3 polyunsaturated fatty acids esters concentration, Revista de Chimie, 66(6), 911-917, 2015, Factor impact: **0,956**, ISSN: 0034-7752;

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N.G. Ștefan, P. Iancu, V. Pleșu, I. Călinescu, N.D. Ignat, Highly efficient deacidification process for *Camelina sativa* crude oil by molecular distillation, Sustainability, Vol.13, Iss.5, 2818, 2021, Factor impact: **2,576**, https://doi.org/10.3390/su13052818;

Patent

Enascuta C.E., Stepan E., Plesu V., Iancu P., **Stefan N.G**., Procedeu de obținere a unui ulei cu conținut mărit de acizi grasi polinesaturați și a unui biocarburant diesel, RO129836/30.03.2017, Universitatea POLITEHNICA din Bucuresti, 2017;

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Ștefan N.G., Pleșu V., Călinescu I., Iancu P., Bonet-Ruiz A., Bonet-Ruiz J., Toma A, Mathematical modelling of molecular distillation, 20th Romanian International Conference on Chemistry and Chemical Engineering (RICCCE20), Poiana Brașov, Brașov, 6-9 Septembrie 2017;

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CHAPTER 6
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