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Optimization of molecular distillation to concentrate ethyl esters of eicosapentaenoic (20:5 ω -3) and docosahexaenoic acids (22:6 ω -3) using simplified phenomenological modeling

Pablo C Rossi,^a* María del C Pramparo,^a María C Gaich,^a Nelson R Grosso^b and Valeria Nepote^b

Abstract

BACKGROUND: Squid oil contains high concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The purpose of this work was to optimize the process of separation by molecular distillation of ω -3 fatty acid ethyl esters obtained from squid oil. The separation process was conducted in two stages in a laboratory-scale molecular distiller. A mathematical model based on the mass transfer phenomena was developed. The Nelder-Mead numerical method was used to optimize the model.

RESULTS: The ω -3 content in the output material of the stage II increased with the temperature of stage I (T^1). The amount of distillated material in stage I increased and the distillated material in the stage II decreased with the increment of T^1 . That implied a decreasing of the ω -3 recovery in the distillated material in the stage II. In addition, the ω -3 recovery increased with the temperature of stage II (T^2), but the temperatures should be less than 140 °C to avoid chemical changes. The optimization results showed an optimal process at $T^1 = 120.5$ °C and $T^2 = 140$ °C.

CONCLUSION: The theoretical model and the optimization give decision criteria about the operative conditions for reaching the highest yield during molecular distillation of ω -3 fatty acid ethyl esters. © 2011 Society of Chemical Industry

Keywords: molecular distillation; EPA; DHA; ω -3; optimization

INTRODUCTION

Fish oil is a rich source of compounds called ω -3 polyunsaturated fatty acids which play an essential role in human diet because of their capacity to prevent diseases. Particularly, two ω -3 fatty acids, 5,8,11,14,17-eicosapentaenoic acid (EPA, 20:5) and 4,7,10,13,16,19-docosahexaenoic acid (DHA, 22:6), are important functional constituents of the human body.¹

Fish oil is obtained as a by-product obtained from the production of fishmeal.² The fish oil containing high percentages of ω -3 has total EPA and DHA concentrations that range from 140 to 300 g kg⁻¹. In general, fish oil is traded as soft gel capsules, aqueous emulsions or mixed with vegetable oils. However, the consumption of these caps is not well accepted by many agencies. Primarily due to the strong organoleptic properties of fish oil which makes the consumer feel the sensation of eating fish.³

The preparation of ω -3 concentrated products could have interest as a nutraceutic. They would allow us to eat fewer total fatty acids and more essential fatty acids. Furthermore, another goal is to obtain fewer compounds that produce fish odor and taste. Products of these characteristics should be more accepted by consumers. The procedure for the preparation of ω -3 concentrated products should focus on three steps: (1) fatty acids form triglycerides, so they have be separated from the glycerol molecule; (2) fatty acids have very similar physicochemical characteristics, which makes their separation more difficult; and (3) they are highly thermo-labile compounds.⁴

Simple fractionation processes do not discriminate between different polyunsaturated fatty acids found in fish oil. Several methods have been developed to prepare enriched fractions in EPA and DHA. However, due to high costs, some methods are only suitable for small-scale preparations. The proper techniques for small-scale preparations include a controlled winterization,⁵

b Universidad Nacional de Córdoba, IMBIV-CONICET, Córdoba, Argentina

^{*} Correspondence to:Pablo C Rossi, Facultad de Ingeniería, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, 5800-Río Cuarto, Córdoba, Argentina. Email: prossi@ing.unrc.edu.ar

Facultad de Ingeniería, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, 5800 Río Cuarto, Córdoba, Argentina

Table 1. Properties of fish oil	
Property	Value
Acidity (g kg ⁻¹ of oleic acid)	250
Peroxide index (meq $O_2 kg^{-1}$)	0.8
Humidity (g kg ⁻¹)	1
Unsaponifiable material (g kg ⁻¹)	27.4
Refraction index (at 21 $^\circ$ C)	1.48
Viscosity (cp at 22 $^{\circ}$ C)	53-57
Density (g dm ⁻³)	929
lodine index (g I_2 kg ⁻¹ of sample)	1766.1

molecular distillation,⁶ fractionation of the urea phase,⁷ and chromatography with silver resin.⁸ Another feasible technique is the extraction using supercritical carbon dioxide but it is extremely expensive.⁹ At commercial production scale, the technique of molecular distillation is currently the most promising technology.

Molecular distillation is useful for the separation, purification and concentration of thermolabile substances with low vapor pressure.^{6,10} This process is based on the separation of components based on their differences in volatility. For that reason, esters of fatty acids have a better separation efficiency than non-esterified fatty acids.¹¹

Several theoretical models have been reported. Cvengros *et al.*¹² have used a theoretical model to analyze the molecular distillation operation for binary mixtures. They studied the influence of some variables involved in the process. Bose and Palmer¹³ reported that concentration and temperature gradients in the liquid phase decreases separation efficiency. Bandarkar and Ferron¹⁴ worked on the mass and heat transfer in a liquid film on a centrifugal evaporator. Batistella and Maciel¹⁵ have provided comparative results of efficiency and performance between falling film and centrifugal molecular distillation equipment.

Theoretical models are multidimensional and non-lineal models. For this reason, it is required to use numerical optimization methods like Gauss–Newton, conjugated gradient, Levenberg–Marquardt, and Nelder–Mead, among others. The Nelder–Mead optimization method guarantees the convergence and has the advantage of being more robust and simpler than the other methods.^{16,17}

The aim of this study was to optimize the process of separation by molecular distillation of ω -3 fatty acid ethyl esters obtained from squid oil using a simplified phenomenological model validated with laboratory experimental tests.

EXPERIMENTAL

Samples

Deodorized oil from the squid *Illex argentinus* was provided by a local industry (Grupo CONARPESA, Caleta Olivia, Santa Cruz, Argentina). This oil was prepared by enzymatic hydrolysis and refining processes.

Characterization of the raw material

In order to determine the characteristics of the raw material and the quality of products, the following analyses were performed by AOCS methods (Table 1): free fatty acids, peroxide value, humidity, unsaponificable matter, refractive index, viscosity, density and iodine value.¹⁸



Figure 1. Gas chromatography of fatty acid ethyl esters.

Analysis of fatty acid ethyl esters

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A sample of marine oil was esterified by alcoholysis reaction using ethanolic solution of KOH (0.4 mol dm^{-3}). After the esterification reaction, the alcohol and lipidic phases were separated.¹⁹ The ethyl ester fatty acids were analyzed on a Hewlett Packard gas-liquid chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) using a capillary column (HP-INNO-Wax, 30 m imes 0.32 mm imes 0.5 nm). The injector temperature was 220 °C. The temperature program was 150 °C for 1 min, rate 5° C min⁻¹ until a temperature of 225 °C, then a rate 5 °C min⁻¹ and a final temperature 260 °C. The carrier gas (nitrogen) had a flow rate of 1 mL min⁻¹. The separated fatty acid ethyl esters were identified by comparing their retention times with those of authentic samples which were purchased from Sigma Chemical Co. (St Louis, MO, USA). Quantitative fatty acids analysis was performed using heptadecanoic acid ethyl ester as internal standard.

Fourteen different compounds were detected by using gas chromatography. The chromatography trace presented in Fig. 1 shows four clearly spaced groups of peaks which made up 700 g kg⁻¹ of the total mixture. These groups of peaks can be characterized by the chain length of similar fatty acids. Therefore, the compounds of chromatography could be grouped into four classes of fatty acids of similar chain length. The remaining 300 g kg⁻¹ is a mixture of organic compounds of high molecular weight; in this case it is defined a new compound, a diglyceride of palmitic and oleic fatty acids which is assumed to have similar properties to the mix. The feed composition was determined with this procedure whose result is shown in Table 2.

Table 2. Feeding oil composition		
Ethyl ester fatty acid	Mass fraction (g kg $^{-1}$)	
Palmitic acid-EE 220		
Oleic acid-EE	180	
EPA-EE	133	
DHA-EE	160	
High-weight compounds	307	



Figure 2. Molecular distillation flow-sheet. MD1, stage 1; MD2, stage 2. TK1, TK2, distillate storage tanks.

Molecular distillation

The equipment used to conduct the experimental activities included a Parr reactor of 600 mL and a KDL4 UIC molecular distiller. The operation of concentration was developed in two consecutive stages of molecular distillation as shown in Fig. 2.

The molecular distillation was developed at a pressure of 40 Pa with a rotor speed of 150 rpm ($1.12 \times g$). The feed temperature was 60 °C. Evaporating temperatures between 100 and 120 °C for the first stage and 120 and 140 °C for the second stage of the process were analyzed. In all cases, the chemical composition of the obtained fractions was determined.

Phenomenological modeling

The transport equations which describe the evaporation process in the film were obtained using the momentum, mass (global and for each component), and energy corresponding balances. The constitutive equations complete the phenomenological modeling. In this case, the Langmuir–Knudsen equation was used for predicting the evaporation rate of each component in the film surface. This equation has been deduced from the kinetic theory of gases.⁵

In order to develop a simplified mathematical model, the following assumptions have been made:

- 1. Steady-state conditions prevail throughout the process.
- 2. A low condensation temperature is maintained to avoid the re-evaporation from the condensation surface.
- 3. A rectangular coordinate system describes the variables of the film (the film thickness is very small compared to the radius of the cylindrical evaporation surface).

- 4. The temperature and radial composition gradients along the equipment are neglected.
- 5. The influence of feed temperature is neglected.

The molar flux density, N_i , for each component was expressed as a function of evaporation rate, k_i , as follows:

$$N_i = x_i^{\mathsf{S}} \cdot k_i \tag{1}$$

where x_i^{S} is the molar fraction of the *i*-component in the evaporation surface.

The evaporation rate of each component, k_i , was evaluated from the Langmuir–Knudsen equation:

$$k_i = \frac{P_{V_i}(T^S)}{\sqrt{2\pi R M_i T^S}}$$
(2)

where T^{S} is the evaporation surface temperature, P_{v_i} is the vapor pressure of the *i*-component, M_i is the molecular weight of the *i*-component, and R is the universal gas constant.

As a result of the implementation of differential mass balances for each component, the respective equations of molar flow variation with the *y*-vertical position were obtained:

$$\frac{\mathrm{d}I_i}{\mathrm{d}y} = -2\pi R_\mathrm{c} k_i x_i^\mathrm{S} \tag{3}$$

where R_c is the radius of the cylindrical surface on which the film descends. The associated boundary condition is presented below:

$$y = 0, \ l_i = l_i^0$$
 (4)

where I_i^0 is the feed molar flow of the *i*-compound.

The total evaporated flow was calculated by the sum of the individual molar flows (of each component):

$$I = \sum_{i=1}^{5} I_i \tag{5}$$

The system of differential equations was solved using the numerical solution trough Runge–Kutta method of four to five orders, using MATLAB 7.8 software.²⁰

Separation efficiency

The separation efficiency, *S*, is a parameter of interest in monitoring the molecular distillation operation. This separation efficiency was defined by the equation:

$$S = \log_{10} \left(\frac{R_D}{R_F} \right) \tag{6}$$

where R_D and R_F are relations of distillate (*D*) and feeding (*F*) compositions, respectively. This relationships are the lightweight compounds (*L*) (palmitic acid ethyl ester (PA-EE) and oleic acid ethyl ester (OA-EE)), divided by the ω -3 compounds (ethyl esters of fatty acids, ω -3 EPA-EE and DHA-EE). To calculate these relationships, the respective mass fractions were used:

$$R_D = \frac{X_{\omega3}^D}{X_L^D}; \quad R_F = \frac{X_{\omega3}^F}{X_L^F} \tag{7}$$

Table 3. Predicted thermodynamic properties of ethyl ester fatty acids

Ethyl ester fatty acid	T_{c} (°C)	<i>P</i> _c , ×10 ⁵ (Pa)	$V_{\rm c} ({\rm m}^3{\rm kmol}^{-1})$	T_{N} (°C)
Palmitic acid-EE	488.06	11.58	1.07	339.04
Oleic acid-EE	516.20	10.56	1.16	367.07
EPA-EE	562.84	10.66	1.19	407.51
DHA-EE	592.84	9.75	1.29	434.11
High-weight compounds	740.60	7.36	1.71	636.85

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Table 4.	Table 4. Experimental results from both molecular distillation stages						
<i>T</i> ¹ ([°] C)	<i>T</i> ² ([°] C)	D^1/F	$X_{\omega 3}^{D^1}$ (g kg ⁻¹)	D^2/F	$X_{\omega 3}^{D^2}$ (g kg ⁻¹)	R_D^2	S
100	120	0.171	163	0.431	399	0.66	-0.043
100	130	0.171	163	0.508	435	0.77	0.022
100	140	0.17	163	0.512	480	0.92	0.100
110	120	0.33	186	0.263	430	0.75	0.012
110	130	0.33	186	0.373	566	1.30	0.250
110	140	0.33	186	0.420	526	1.11	0.181
120	120	0.461	245	0.125	603	1.52	0.317
120	130	0.461	245	0.189	679	2.12	0.461
120	140	0.461	245	0.216	681	2.14	0.466

Prediction of the properties of fatty acid ethyl esters

In order to calculate physical and thermodynamic properties of pure compounds, correlations available in the literature were used. Specifically, the model required the vapor pressure, the density of the pure components in liquid phase, and the density of the liquid mixture.

Benson's procedure was selected to calculate densities of esters.²⁰ The method of Frost-Kalkwarf-Thode²⁰ was the most appropriate to obtain the vapor pressure. Both properties were obtained as a function of temperature.

For the determination of these properties, critical properties and the normal boiling point of each component were required. With respect to critical properties, the method of fragmentation by group contribution applied to organic compounds was used;²¹ while the method of Lydersen-Forman-Thode²⁰ was used to calculate normal boiling point. Values estimated from these correlations are shown in Table 3.

The following equation was used in the calculation of vapor pressure of the pure compounds:

$$\log(P_{v_r}) = \left(\frac{B}{T_c}\right) \left(\frac{1}{T_r} - 1\right) + \left(\frac{1.80B}{T_c} + 2.67\right) \log T_r + 0.1832 \left(\frac{P_{v_r}}{T_r^2} - 1\right)$$
(8)

where P_{vr} is the reduced vapor pressure, T_c is the critical temperature, T_r is the reduced temperature and B is an adjustment parameter to be calculated with any known point such as normal boiling temperature.

The equation used to calculate densities in the liquid phase for the components ρ_i was:

$$\rho_{i} = 2\rho_{c} + \frac{(T_{c} - T)}{T_{c} - T_{N}}(\rho_{N} - 2\rho_{c}) - \rho_{v}$$
(9)

where the critical (c) and normal (N) densities were calculated as:

$$\rho_{\rm c} = \frac{M}{\hat{V}_{\rm c}} \quad \text{and} \quad \rho_{\rm N} = \frac{M}{\hat{V}_{\rm N}}$$
(10)

The vapor density, $\rho_{\rm V}$ is determined by the ideal gas equation and the normal volume \hat{V}_{N} is calculated according to the equation:

$$\hat{V}_{\rm N} = \frac{\hat{V}_{\rm c}}{0.422 \times \log(P_{\rm c}/101325} + 1.981$$
(11)

where P_c is the critical pressure.

Optimization process

The objective for the optimization of the molecular distillation process was to concentrate in ω -3 fatty acid ethyl esthers every distilled fraction as much as possible. In the distillate from stage I (D^1) we pretended to obtain the highest recovery rate and mass fraction of 'light' compounds (palmitic and oleic acid ethyl esters). In the distillate of stage II (D^2) , we wished to obtain the highest recovery rate and mass fraction of ω -3 fatty acid ethyl esters (EPA-EE and DHA-EE). For this optimization, the objective function (OF) was maximized as follows:

OF =
$$w_1 \operatorname{Re} c_{\omega 3} + w_2 X_{\omega 3}^{D^2} + w_3 \operatorname{Re} c_L + w_4 X_L^{D^1}$$
 (12)

where w_i is the proposed weights to each parameter, $X_i^{D^i}$ is the mass fraction and Rec_i is the respective recoveries calculated by:

$$\operatorname{Re} c_{\omega 3} = \frac{X_{\omega 3}^{D^2}}{X_{\omega 3}^{F}} \cdot \frac{D^2}{F}$$
(13)

and

$$\operatorname{Re} \mathsf{c}_{L} = \frac{X_{L}^{D^{1}}}{X_{L}^{F}} \cdot \frac{D^{1}}{F} \tag{14}$$

The optimization was obtained through the implementation of numerical methods (Nelder-Mead) for the generated nonlinear multidimensional problem.²²

Table 5. Comparison between theoretical and experimental results							
		D^2/F			$X^{D^2}_{\omega 3}$ (g	kg ⁻¹)	
<i>T</i> ¹ (°C)	<i>T</i> ² ([°] C)	Experimental	Theoretical	Error	Experimental	Theoretical	Error
100	120	0.431	0.42800	0.01	399	383	0.04
100	130	0.508	0.51952	0.02	435	472	0.08
100	140	0.512	0.53884	0.05	480	491	0.02
110	120	0.263	0.29405	0.12	430	455	0.06
110	130	0.373	0.37309	0.00	566	547	0.03
110	140	0.420	0.39767	0.05	526	575	0.09
120	120	0.125	0.12697	0.02	603	653	0.08
120	130	0.189	0.17575	0.07	679	729	0.07
120	140	0.216	0.20115	0.07	681	762	0.12

RESULTS AND DISCUSSION

Laboratory experimental results

 ω -3 fatty acid ethyl esters are compounds with intermediate volatility in the esterified fish oil. So, two-stage molecular distillation was performed to separate more and less volatile compounds than the ω -3 fatty acid ethyl esters in the first and second stages, respectively. Experimental results and the corresponding separation efficiency, *S*, are shown in Table 4. The negative value of the separation efficiency (*S*) was caused by a disadvantageous operational condition where the final product was more diluted in ω -3 fatty acid ethyl esters than the feeding ones. Based on the small difference in separation efficiency at high temperatures, the study of the behavior at higher temperatures was analyzed using phenomenological modeling.

Experimental validation of the model

Table 5 compares experimental D^2/F and $(X_{\omega_3}^{D^2})$ with theoretical ones for validating the proposed model. The relative error in each variable was calculated using the equation:

$$error = \frac{|approximate value - exact value|}{|exact value|}$$
(15)

The low errors revealed that the values predicted by the model were a good approximation of experimental results. The resulting model had a good response in ω -3 concentration and flow ratio changes due to temperature change in every stage.

The system behavior at different temperature in each stage is shown in Table 5. The $\omega\text{-}3$ content in the output material of stage II $(X_{\omega_3}^{D^2})$ increased with the temperature of stage I (T^1) . This was a consequence of higher separation of the lightweight compounds that were not required in the stage II. In addition, the amount of distilled material in stage I increased and the distilled material in stage II decreased with the increment of T^1 . That implied a decrease of the ω -3 recovery in the distilled material in stage II (D^2/F) . Considering this behavior, it was necessary to determine the optimal condition between ω -3 purity and the recovered amount. With respect to the temperature at stage II, the ω -3 recovery increased with temperature (T^2). The temperature for ω -3 distillation should be less than 140 $^{\circ}$ C to avoid chemical changes of thermolabile compounds.⁴ The evaporation temperatures to separate ω -3 fatty acid ethyl esters were predicted by the model and validated experimentally. Those temperatures were similar to those found by other authors. Liang

Table 6.	Optimization results for ω -3 fatty acid ethyl ester molecular
distillation	by two stages at $T^1 = 120.5$ °C and $T^2 = 140$ °C

Variable	Value
Recovery of light compounds at stage I, Rec	0.915
Composition of light compounds at stage I, $X_L^{D^1}$ (g kg ⁻¹)	708
Recovery of EPA+DHA from stage II, Rec_{ω^3}	0.478
Composition of EPA+DHA at stage II, $X_{\omega 3}^{D^2}$ (g kg ⁻¹)	804

and Hwang⁴ determined elimination curves of esterified squid oil by multiple stage molecular distillation at different temperatures ranging from 50 to 150 °C. At temperatures less than 130 °C, the final product had a higher concentration of light compounds (fatty acids <18 carbons). At temperatures between 130 and 150 °C, the final product had higher EPA and DHA concentrations.

Optimization results

The optimization results showed an optimal process at $T^1 = 120.5$ °C and $T^2 = 140$ °C. The surface plot presented in Fig. 3 had the restricted peak at $T^2 = 140$ °C. This represents a maximum temperature to avoid thermal decomposition of the components involved.⁵ The contour plot of the model is shown in Fig. 4.

Although the optimal value was outside of the studied experimental ranges, the validity can be considered acceptable due to the fact that it was very close (0.5 °C) to the researched area. Table 6 shows the final results of optimization considering the four variables to optimize. These results corresponded to equal weights, w_i , for each variable (0.25) to limit the objective function to between 0 and 1.¹⁶ These results can be modified by weighting one interest variable more than another for each particular application.

The maximizing point of the objective function led to a high value of ω -3 composition in the distillate of stage II but the ω -3 recuperation with respect to the feeding obtained (Rec ω ₃) was low. These losses in ω -3 ethyl ester were due to the material distilled at stage I, residual material from stage II and non-esterified fatty acids remaining during the esterification process. Although this product was highly concentrated in ω -3 and it is possible to produce it technologically, the economic feasibility should be carefully considered due to the high operative costs.

According to Liang and Hwang,⁴ the separation of squid visceral oil ethyl esters with a 10 $^{\circ}$ C range gave higher concentrations of EPA and DHA than with a 20 $^{\circ}$ C interval. Nevertheless, from an



Figure 3. Surface of the objective function to maximize the objective function (OF) depending on the temperature of evaporation of stages I and II ($w_i = 0.25$).



Figure 4. Level contours of the objective function (OF) based on evaporation temperatures of stages I and II.

economical point of view, it is preferable to carry out distillation at 20 $^{\circ}$ C intervals. In the present work, we observed that higher concentrations of EPA and DHA ethyl esters were reached when working in optimum conditions with a range of 20 $^{\circ}$ C between stages I and II.

In the study by Liang and Hwang,⁴ and in this work, it was also observed that the concentrations of ω -3 ethyl esters increased but their yield decreased with the number of distillation stages.

Finally, it is important to feed enriched in EPA and DHA with raw material in order to reach higher concentrations of ω -3 ethyl esters in the distilled fraction.⁴

CONCLUSIONS

A mathematical model has been developed which allowed optimizing the molecular distillation process and consequently

seeking the conditions that produce good recovery of ω -3 fatty acid ethyl esters with a high purity degree. The model also allowed an analysis of the influence of different operating variables.

The model was experimentally verified, obtaining a good agreement between experimental results and predicted values. This verification confirms the validity of the theoretical model developed and also confirms the good estimation of physicochemical properties.

This method of analysis allows us to know the influence of operating temperature at every stage in the overall efficiency of the process.

The theoretical model and the optimization give a criterion for making decisions about the operative conditions for reaching the highest yield during molecular distillation of ω -3 fatty acid ethyl esters.

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Variable B Definition Adjustment parameter of the vapor pressure model Unit(s) 1 Dist T and Hwang Y, n2 Faty acids modulate the progression of antitis. Inform 11:1410–414 (2000). Valenzuela A, Nieto S and Lauy, R acids modulate the progression of antitis. Inform 11:1410–414 (2000). D' Flow of the distilled stream (based parameter of the yaporation rate of the t-component Ni kg s ⁻¹ 3 Tartse C, Nettekown T and Hill C, Pacefaot technologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de Oro & A&G., 10 Aniversanios de uso adimenticio y farmacologico. Libro de S., 10 Aniversanio the A., 10 Aniversanio the A., 10 Aniversanios d	Nomenclat	ure		REFERENCES
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