Extraction and characterization of *Moringa oleifera* Lam var. Supergenius seed oil from Cuba

Yosvany Díaz-Domínguez, Danger Tabio-García, Maylin Rondón-Macias, Elina Fernández-Santana, Susana Muñoz - Rodríguez, José María Ameneiros - Martínez, Ramón Piloto-Rodríguez.*

Universidad Tecnológica de la Habana "José Antonio. Echeverría", Facultad de Ingeniería Química, 19390, La Habana, Cuba. ydiaz@quimica.cujae.edu.cu.*Universidad Tecnológica de la Habana "José Antonio. Echeverría", Facultad de Ingeniería Mecánica, 19390, La Habana, Cuba

Recibido: 14 de enero de 2016. Aceptado: 24 de febrero de 2016.

Palabras clave: *Moringa oleifera*, aceite de moringa, propiedades de aceite, proceso de extracción, reología
Key words: *Moringa oleifera*, moringa oil, oil properties, extraction process, rheology

RESUMEN. La *Moringa (M.) oleifera* es altamente valorada a nivel mundial por las propiedades que posee. Una de las partes aprovechables del árbol es la semilla, ya que estas contienen entre un 30 y 45 % de aceite. El presente trabajo tiene como objetivo fundamental desarrollar la extracción de aceite a partir de las semillas de *M. oleifera*, variedad (var.) Supergenius de origen cubano mediante el empleo de hexano como disolvente. El porcentaje de extracción de aceite se estableció como variable respuesta en un diseño experimental del tipo factorial 2^2, donde la relación soluto-solvente y el tiempo de extracción fueron los factores estudiados a dos niveles. Ambos factores influyeron de manera significativa en la variable respuesta, (con valores) asociados de $P < 0{,}05$. El modelo matemático obtenido explicó el comportamiento de la variable respuesta en 91,45 %. A partir del comportamiento del porcentaje de extracción de aceite con el tiempo de extracción, se propone un reajuste en dicho tiempo, garantizando un ahorro de energía. Las propiedades físico químicas y el perfil de ácidos grasos del aceite extraído están en correspondencia con reportes hechos para otras variedades de *M. oleifera*. Solamente existieron diferencias en cuanto al índice de acidez. El comportamiento reológico demostró que el aceite de *M. oleifera* es Newtoniano.

ABSTRACT. *Moringa* (*M.*) *oleifera* is highly valued worldwide because of its properties. The seed is one of the usable parts of the tree because of its oil content between 30 % and 45 %. This paper aims to develop the oil extraction from seeds of *M. oleifera*, Supergenius variety (var.) of Cuban origin using hexane as solvent. The extraction percentaje was set as the response variable in the factorial 2^2 experimental design. The solute-solvent ratio and the extraction time were the studied factors at two levels. Both factors significantly influenced the response variable, with associated P values less than 0.05. The obtained mathematical model explained the behavior of the response variable at 91.45 %. An adjustment of the extraction time was developed in order to ensure energy saving. Physical and chemical characteristics and the fatty acid profile of the extracted oil agree with several reports of *M. oleifera* varieties. The difference is shown in terms of the acid content value. The rheological behavior of *M. oleifera* var. Supergenius oil was found to be Newtonian in nature.

INTRODUCTION

The trees of *Moringa* grow mainly on semi-arid zones, but it develops better in dry sandy soils. The growth is fast and resistant to drought. The tree is native of southern foothills of Himalaya mountain range.[1] It can raise to a height that can oscillate between 5 and 10 m, sometimes even 15 m. It can flower and develop fruits in one year. *M. oleifera* tree is considered one of the most useful trees of the world, due to almost all parts of the tree are used. In the tropics, it is used as foliage for the cattle.[2] The foods extracted from the plant have a high nutritive value.[3,4]

The root, the stem, the leaves, the flowers, the fruits and the seeds constitute the plant. The root, stem and the bark are a potential source of antioxidant- compounds. The leaves and the seeds are of high interest.[5] These seeds are heavily-used because of their oil content. Seeds are fleshy, covered by a fine shell of coffee color. They have three wings, or winged seeds from 2.5 to 3 mm in length. When the shell is taken away, the endosperm is discovered. It is whitish and very oleaginous.[6]

The study of *M. oleifera* seeds is important because it is a vegetal specie with high oil content (30 - 45 %). This oil has several well-known worldwide uses; among these it is possible to mention its potential like biofuel, specifically to produce biodiesel[7-9] for lubricating oil and cosmetics applications.[6,10] These applications demonstrate their lofty cost-reducing value. Oil has a high content of monounsaturated or polyunsaturated fatty acids,[11] a very important quality for lipids transformation in the organism.[4] *M. oleifera* oil, also known as oil of Behen, contains an important percentage of oleic acid (around 70 %). Oleic acid is a monounsaturated fatty acid that confers a great stability to this oil in front of oxidation. For such a motive, moringa's oil is more stable than the canola oil, soja bean oil and palm tree oil.[4]

In Cuba, a national program of Moringa culture is developed. The Agricultural authorities have taken interest in Moringa disseminating and use. It has been established as the first rate resource to insure human and animal feeding. Today, Moringa cultivation has reached several provinces into the country. That is the reason of the availability of a great deal of seeds. There are reports of physical – chemical characterization and fatty acid profile for Cuban *M. oleifera* seed oil.[12,13] However these studies do not detail the *M. oleifera* variety. Due it is still unknown the physical-chemical characterization, the fatty acid composition and rheological behavior of *M. oleifera* var. Supergenius seed's oil. The goal of the present paper is to develop the extraction and the characterization of Cuban *M. oleifera* var. Supergenius seeds oil.

MATERIALS AND METHODS

Seeds of *M. oleifera*

The variety of seed of *M. oleifera* Lam used was Supergenius. The seeds were provided by The International Health Center La Pradera (23º 72' 0'' N, 82º 23' 0'' W, La Havana, Cuba).

Solvent applied in the solid liquid extraction

Hexane (boiling point: 68.7 ºC, specific gravity: 0.659, refractive index: 1.375 and purity > 98 %).

Seeds preparation

Seeds were prepared for the extraction process, by separating firstly the cotyledons from the peelings (exerting manually pressure on them). Later, the seeds were placed into a mortar lab to be crushed in order to diminish their particle size. The contact area between seeds and solvent during the oil extraction process was increased in this way. As a complement, the crushed seeds were sieved according to 631 Cuban standard[14] for knowing the particle size distribution of seeds after trituration.

Drying of crushed seeds

Seeds were dried for two hours at 55 ºC in a stove (model DHG 916A, Germany). Temperature should not exceed 60 ºC to avoid protein denaturalization.

Stage of extraction

Soxhlet method was applied to the process of extraction. After the seeds preparation for the extraction, a mass of 10 g was weighed (using an analytical balance Sartorius BS 124S, Germany). This mass was introduced into a thimble (porous cartridge to be introduced into the Soxhlet apparatus, in a way that seeds cannot get out of this). The hexane was added according solute solvent ratio previously defined to develop the research. The experimental design specifies them. The matched condenser has water like refrigerant. In order to guarantee the operation, an electric iron was used as means of heating and temperature was regulated (boiling temperature of the solvent, 69 ℃).

Stage of separation of the oil

An evaporation process was applied to the mixture of oil and solvent in order to remove one from the other. The process was developed in a rotary evaporator (IKA-WERK HB 4 basic, Germany). The oil product was weighted in a scale to calculate the oil mass percentage of extraction by means of equation 1:

$$Extraction_{oil}(\%) = \frac{mass_{oil}}{mass_{seed}} * 100 \tag{1}$$

Experimental design

Multilevel factorial design, 2^k, where k = 2, taking two factors into account at two levels. The considered factors were the solute/solvent ratio and time of extraction. This last was introduced due there were no previous background about the process kinetics. The fixed levels were in the rank of 1:4 and 1:6 for solute/solvent ratio. They were measured in mass of seeds and volume of solvent. Analyzed time of extraction was in the interval of 4 to 6 h. In all cases, the response variable was the oil percentage of extraction. For all the experiments, two replies were done. Those factors were defined into the statistical software *Statgraphics Centurion XV* to create an orthogonal matrix with coded values. Coded values were assigned to the levels of the factors (Table 1).

Table 1. Real and coded values for variables involved at the experimental design

Factors	Real Value	Coded Value
Solute solvent ratio X_1 (g/mL)	1:4	-1
	1:6	1
Time of extraction X_2 (h)	4	-1
	6	1

Physical-chemical characterization of the extracted oil

Determinations of saponification value, and iodine, refractive, acidity and peroxide indexes of the extracted oil were made using standard analytical methods.[15] The composition of fatty acids was developed according AOCS, Ce 1b-89.[16] The fatty acids content in the oil was determined by gas chromatograph and mass spectrometer (GC-MS) of the previously methylated oils. The gas chromatograph was Agilent Technologies 6890N (G1530N) coupled with a Agilent 5975 Mass Selective Detector (MSD), Network GC System with a SP 2560 column, 100 m; injector cold on column and 1 mL/min of helium as carrier gas. The detector used for quantification was a Flame Ionization Detector operated at 250 °C, 300 mL air/min and 30 mL H_2/min. Make-up gas: He 20 mL/min. Temperature program: 100 °C (1 min), 4.8 °C/min-150 °C, 1°C/min, 170 °C (48 min), 1 °C/min, 174 °C (30 min), 5 °C/min, 240 °C (10 min). The fatty acids were identified by comparing the retention times and their mass spectra with a mass spectra data library. The Mass Selective Detector column and temperature program were the same used for GC-FID. The ion source and interface temperature were set to 200 °C. All the samples were analyzed in scan mode (m/z = 50 - 450). The ionization energy was 70 eV and Agilent MSD Productivity ChemStation software was used.

Rheological behavior

The rheological behavior of *M. oleifera* oil was evaluated by the dynamic viscosity using a Antón Paar Physica rheometer MCR-301 model with Peltier heating system. The experiment was carried out with shear rate range of 0.001-1 000 s^{-1} and temperature at 40 ºC. The data were obtaining from Rheoplus (3.21) software.

RESULTS AND DISCUSSION

Analysis of sieving

The sieving analysis allowed getting the distribution of present size of particles in the seeds after the trituration process (Table 2).

Table 2. Sieving analysis results

Class number	Sifter Diameter (mm)	Retained Mass (g)
1	2.00	4.5
2	1.60	2.1
3	1.40	1.0
4	1.00	3.1
5	0.80	0.1
6	0.63	0.6
7	0.50	0.6
8	0.40	0.5
9	0.31	0.6
10	0.20	0.6
11	0.16	0.4
12	0.12	0.0
		∑=14.1

From this information, granulometric distribution graphic of crushed seeds was built (Fig. 1).

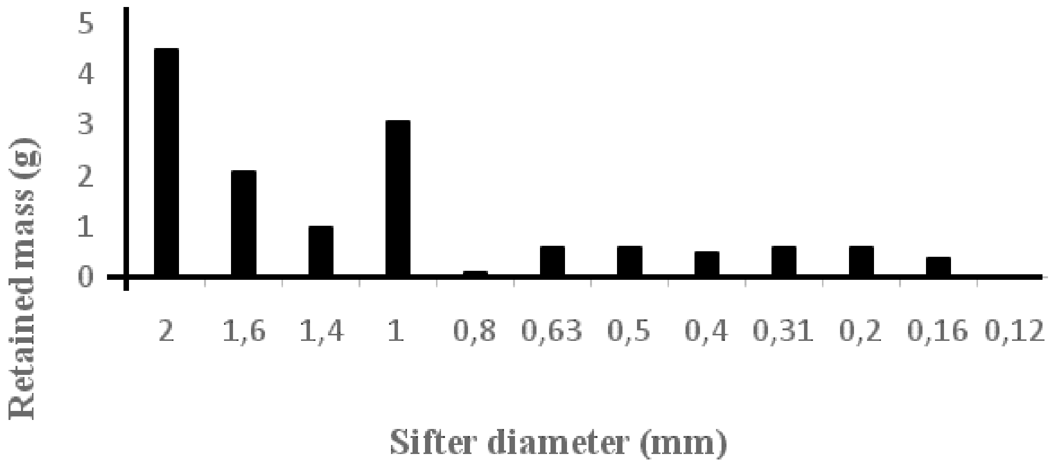

Fig. 1. Granulometric distribution of crushed seeds.

The analyzed sample is a non-uniform particle size distribution (Fig 1). Most of them are concentrated between the sifters of bigger opening (from 2 mm to 1 mm). 76 % of the total screened mass was retained in the aforementioned sifters. The particle size can influence the velocity of the extraction and the operation yield in different manners. A decrement in particle size increases the contact area between the solid and the solvent.[2] Mani[10] reports extractions from seeds of Periyakulam 1 (PKM 1) variety of Georgia, with several solvents. Particle size was one of the variables included at the experimental design. The studied values were 2.2; 1.7; 1.0; 0.3 and 0.18 mm. The optimal value was 0.62 mm. From the reports made for Periyakulam 1 (PKM 1) variety of Georgia, it is necessary to study Supergenius variety in order to find out about the influence of the particle size (considered as a factor) in the oil extraction process.

Oil Extraction

The extraction of oil was developed according to the description exposed in materials and methods. The quantities of extracted oil and the respective percentages of extraction were calculated through the equation 1 (Table 3).

Table 3. Oil Extraction percentages at the experimental conditions

Solute–solvent ratio	Time (h)	Extracted oil mass (g)	Oil extraction Percentage (%)
1:4	4	3.086 6	30.00
1:4	4	2.924 8	28.90
1:4	4	3.034 3	30.00
1:4	6	3.292 4	32.30
1:4	6	3.164 4	31.10
1:4	6	3.247 4	31.80
1:6	4	3.444 8	32.90
1:6	4	3.201 1	31.40
1:6	4	3.307 9	32.30
1:6	6	3.464 9	33.90
1:6	6	3.654 7	35.70
1:6	6	3.752 7	36.70

The reported percentages of oil correspond to the literature records for different varieties of Moringa. They oscillate generally in the rank of 30 to 45 %.[7] There are not previous references of Supergenius variety to establish a comparative specific opinion. There is only an experiment with solute solvent ratio of (1:4) and four hours of extraction with an extraction percentage underneath 30 % (Table 2). The mean value of the extraction percentage was 31.60 % with a standard deviation of 2.40. There are reports of the process of oil extraction with *M. oleifera* Lam, Concanensis variety of Pakistan. One of the applied alternatives was the use of hexane as solvent, getting extraction percentages around 38.40 %.[17] The highest percentages of extraction were obtained for variety Periyakulam 1 (PKM 1) of Georgia, around 33.10 % achieved precisely with hexane.[10] Efeovbokhan[11] reports percentages of extraction of oil with hexane of 34.71% for *M. oleifera* in Oyo state (west of Nigeria). Martín[12] reports an extraction percentage of 38.1 % (using hexane) for *M. oleifera* from Las Tunas (Cuba), however the variety was not detailed.

If the mentioned results for Supergenius variety are compared with the previous results, these are lightly inferiors to the ones of variety PKM 1 and to the variety developed in the west of Nigeria. However, this difference increases regarding variety Concanensis of Pakistan. In spite of the difference of varieties and the experimental conditions established in this investigation, there are factors like the weather and the geological conditions of the region that can fall into the attained results.

Experimental design

The developed experimental design 2^2, with two replies at each point, enabled the obtaining of a coded model. It explains the behavior of oil extraction from seeds (response variable), considering the studied conditions. The model is represented by equation 2:

$$Extraction_{oil}(\%) = 32.2654 + 1.5797\,X_1 + 1.3301\,X_2 + 0.2841\,X_1X_2 \quad \textbf{(2)}$$

Where:

X_1: Solute/solvent Ratio

X_2: Time of extraction

The statistical parameter R Squared (R^2) points out that the adjusted model explains 91.45 % of the variability in the percentage of extraction of oil. Factors are analyzed from the design, where the variability of the percentage of extraction of oil has been partitioned for each one. This way, statistical significance of every factor is proved by a comparison between its average square and an estimate of the experimental error (Table 4).

Table 4. Analysis of variance for the response variable "Oil Extraction"

Source	Square sum	Freedom degrees	P-Value
A: Solute/solvent Ratio	29.946 1	1	0.001 0*
B: Time of Extraction	21.230 5	1	0.002 4*
AB	0.968 6	1	0.324 1
Total error	5.039 65	6	

In this case, two factors have a probability less than 0.05. This indicates they are significantly unlike zero with a confidence level of the 95 %. The two factors, time of extraction and solute/solvent ratio at the levels established in this study, fell into the response variable in a positive way. However, the analysis demonstrates that interaction between both is not significant because its P-value is higher than 0.05. The finally obtained model is represented by the equation 3, where the interaction between the two factors is excluded.

$$Extraction_{oil}(\%) = 32.2654 + 1.5797\, X_1 + 1.3301\, X_2 \quad (3)$$

From this analysis of variance for the response variable (percentage of oil extraction) the value of Durbin Watson coefficient is obtained. This statistic indicator allows checking if errors are independent because this is a request for the application of the statistical method. The desired value should be around 2 and this happens when probability is larger than 0.05. In this case the Durbin Watson value is 1.744 95 with an associated probability of P = 0.187 0. That means there is not any correlation in errors, or in other words, they are independent. This mentioned model can be accepted to explain the variability of the oil extraction percentage at the case-study conditions. Besides as it started up with an orthogonal design, the coefficient of interactions of the factors can be excluded without any modification in other terms since it did not have influence of significant way in the response variable. From the statistical program, the best value (within the studied conditions) can be acquainted. The best value of the response variable is achieved at the bigger time of extraction (6 h) and solute/solvent ratio of 1:6.

Time of Extraction behavior

Another aspect to take into account is that the seeds of Moringa are considered oil plant seeds with high content of oil. In a process of extraction like the one analyzed here, a great deal of the product of concern is extracted during the first hours, and in an upper moment the oil extraction percentage increases too little. The behavior of the process for *M. oleifera*, var. Supergenius and Cuban origin is unknown. The results for oil extraction percentages at solute/solvent ratio 1:6 related to the time of extraction are plotted (Fig. 2), where the kinetics behavior is shown.

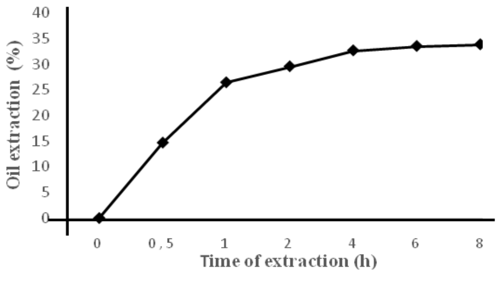

Fig. 2. Influence of time of extraction on the response variable.

As it is observed, the curve shows an asymptotic behavior from times of extraction of four hours and upper (Fig. 2). This indicates that from this time on, the quantities of extracted oil will be very low. So, it could be recommended for the future research with Supergenius variety, to set this time as the time of extraction. This would not affect the oil percentage extraction and would maintain a low energetic cost.

Physico-chemical characterization of extracted oil

The physical-chemical properties of extracted oil from *M. oleifera*, var. The results Supergenius of Cuba, have been compared with some varieties reported in literature.[18] (Tabla 5)

Table 5. Physical-chemical properties of extracted moringa oil

Property	*M. oleifera* Supergenius (Cuba)	Literature				
		M. oleifera Wild (NWFP, Pakistán)	*M. oleifera* Sindh (Pakistán)	*M. oleifera* Periyakulam (India)	*M. oleifera* Mbololo (Kenya)	*M. oleifera* Wild (Malawi)
Iodine Index (g de I/100 g of oil)	64.49 ± 1.81	68.63	69.45	65.58	66.83	65.74
Refractive Index (40 °C)	1.463 0 ± 0.003 0	1.457 1	1.460 8	1.457 0	1.454 9	1.455 9
Density (g/cm^3) 24°C	0.903 0 ± 0.002 0	0.903 2	0.907 0	0.909 0	0.880 0	0.888 0
Saponification Index (mg KOH/g oil)	177.467 ± 1.193	181.4	186.67	188.36	178.11	184.16
Peroxide Index (meq O_2/kg oil)	1.57 ± 0.213	1.27	0.59	1.83	1.80	0.23
Acidity Index (% of oleic acid)	0.70 ± 0.01	0.81	0.40	1.12	0.85	0.82

The indexes of iodine, refraction, saponification and the density obtained for the oil drawn from Supergenius variety are similar to the ones previously reported for *M. oleifera* of Pakistan, India, Kenya and Malawi (Table 5).[18] The iodine index value is 64.49 Iodine g/oil g, which indicates this oil is a sort of monounsaturated (oil in which a monounsaturated fatty acid predominates). The acid number evidences an average value of 0.70 % (oleic acid percentage) with a standard deviation of 0.01, so it is possible to express that there is a low content of free fatty acids in the aforementioned oil. This value was lower than four of the five varieties of *M. oleifera* reported (Table 5). Regarding the peroxide index value, 1.57 O_2 meq/kg oil, it shows not much degree of oil oxidation, being considered an important result to take into account for its storage. The acceptance limit to this property is 5 O_2 meq/kg oil. According to the fatty acid profile, the composition of the extracted oil is obtained (Table 6).

Table 6. Composition of Extracted oil from *M. oleifera*

Fatty Acids	%
C16:0	6.017
C16:1	1.551
C18:0	3.780
C18:1c	75.735
C18:2 n-6	0.734
C18:3n-3	0.161
C20:0	2.489
C20:1	2.319
C22:0	5.474
C:22:1	0.013
C24:0	1.027
Others	0.700

It is observed the total saturated- fatty acids percentage (Table 6). They are palmitic (C16:0), stearic (C18:0), arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0) was of 18.787 %. The bigger percentage was the one for palmitic acid, (6.017 %). The presence of the behenic acid (5.474 %) explains why the *M. oleifera* oil is also named Behen's oil.

M. oleifera's extracted oil shows 79.618 % content of monounsaturated fatty acids, where the oleic acid (C18:1) with a 75.735 % predominated (Table 6). This result corresponds to the achieved results of the iodine index analysis. This is another demonstration that this oil classifies as oil with predominant content of a monounsaturated fatty acid. It is possible to find the composition of *M. oleifera* oil of different varieties (Table 7).[18] In this varieties, the lignoceric acid was not detected (C24:0). It is a first difference with the oil of *M. oleifera*, var. Supergenius, studied in the present paper. The content of saturated fatty acids of oil extracted from Supergenius variety is similar to the one of Mbololo variety of Kenya (19.51 %) and it is lightly lower than the rest of varieties. The bigger difference in this last analysis was addressed, fundamentally, to the composition of stearic acid. For Supergenius variety was 3.78 %, lower than 5.5 0 % of the variety NFWP (Pakistan), 5.67 % of the Sindh (Pakistan), 5.88 % of the Periyakulam (India) and 5.86 % of the Wild (Malawi). That difference is attributable to varieties differences.

Table 7. Fatty acids composition of several Moringa *oleifera* variety[18]

Fatty acids	*M. oleifera* Wild (NWFP, Pakistan)	*M. oleifera* (Sindh, Pakistan)	*M. oleifera* var. Periyakulan (India)	*M. oleifera* var. Mbololo (Kenya)	*M. oleifera* var. Wild (Malawi)
C16:0	6.45	6.50	6.46	6.04	5.51
C16:1	0.97	1.00	1.36	1.46	1.10
C18:0	5.50	5.67	5.88	4.14	5.86
C18:1	73.22	76.00	71.21	73.6	67.79
C18:2	1.27	1.29	0.65	0.73	0.71
C18:3	0.30	Not detected	0.18	0.22	0.21
C20:0	4.08	3.00	3.62	2.76	3.78
C20:1	1.68	1.20	2.22	2.40	2.60
C22:0	6.16	5.00	6.41	6.73	6.81
C22:1	Not detected	Not detected	0.12	0.14	0.11

Also it can be observed that in all the varieties, the oleic acid (67-78 %) predominates (Table 7), so there is a predominance of unsaturated acids (fundamentally monounsaturated) just like in Supergenius variety. Martín[12] and Marrero[13] reports similar results.

There is only a difference with Wild variety, of Malawi. The high content of this fatty acid makes the fatty acid composition profile of *M. oleifera*'s oil similar to the one of olive oil (oleic acid (53-86 %)).[19] This similarity between both oils does not refer to oleic acid only, but also there is correspondence with the composition of palmitic (7-20 %) and linoleic acid (0-3 %).[19]

Viscosity and rheological behavior of *M. oleifera* oil. The viscosity of *M. oleifera* oil was 36,90 mPa.s. These results agree with those reported for *M. oleifera* samples collected in Pakistan. The rheological behavior of *M. oleifera* oil are presented (Figures 3 A and B). It can be observed from the fluency curve a typical linear relationship between shear stress and shear rate (Fig. 3 A). The straight-line indicates that the viscosity of this oil at 40 ºC is independent of the shear rate and thus a Newtonian behavior. In addition, the independence of viscosity on shear rate in the viscosity curve further confirms the behavior (Fig. 3 B). This result is in accordance with the literature.[20]

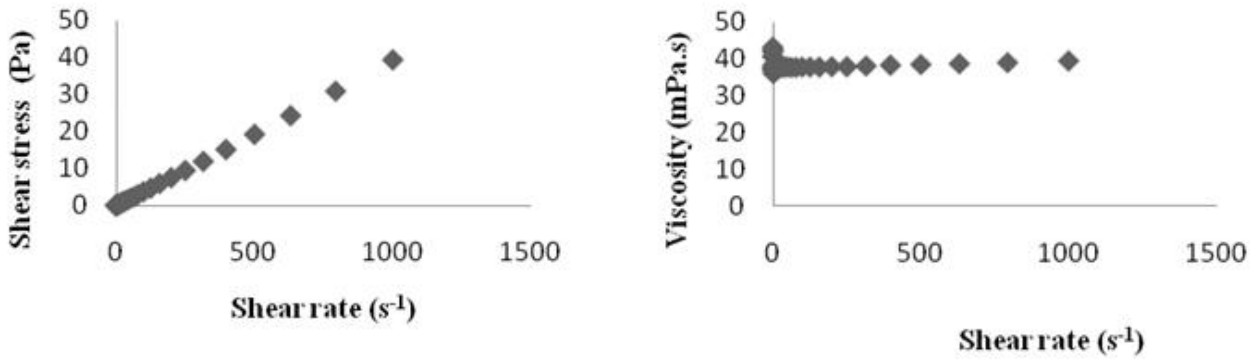

Fig. 3 A and B. Shear stress and viscosity versus shear rate of *M. oleifera* oil at 40 ºC.

CONCLUSIONS

The average oil percentage of extraction from *M. oleifera*'s seeds Supergenius variety of Cuban origin was 31.60%. The factors time of extraction (4 and 6 h) and solute/solvent ratio (1:4 and 1:6) have a significant influence in the oil extraction process. The coded mathematical model, the result of the developed experimental design, explains the variability of the percentage of oil extraction in 91.45 % of cases. The best condition for the extraction was at 6 h with a solute solvent ratio of (1:6). However, the time of extraction study demonstrates it is possible to readjust this variable in 4 H without any important variation in the oil extraction percentages. Physical-chemical characteristics of the extracted oil are similar to the previous reports studied in the specialized literature. The rheological behavior of *M. oleifera* oil was found to be Newtonian. According to the oil yield, the physical-chemical characteristics and rheological behavior, *M. oleifera*, var. Supergenius oil is a potential feedstock for biodiesel production.

BIBLIOGRAPHIC REFERENCES

1. Rodríguez JR, Sánchez DI, López J, Núñez JA, Sánchez RG, Correa MA. Moringa *oleifera* seed extract in the clarification of surface waters. International Journal of Environmental Protection. 2012;2(11):17-21.

2. Abdulkareen AS, Afolabi AS, Awenebe OL. Renewable Energy Sources. Chapter 12, Extraction and optimization ofoil from Moringa *oleifera* seed as alternative feedstock for the production of biodiesel. Rijeka, Shanghai: In Tech.:2011:p.243-268.

3. Amaglo NK, Bennett RN, Curto RB, Rosa EA, Turco VL, Giuffrida A, *et al*. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree Moringa *oleifera* L., grown in Ghana. Food Chemical. 2010;122(4):1047-54.

4. Nguyen HN, Gaspillo PD, Maridable JB, Malaluan RM, Hinode H, Salim C, *et al*. Extraction of oil from Moringa *oleifera* Kernels using supercritical carbon dioxide with ethanol for pretreatment: optimization of extraction process. Chemical Engineering and Processing. 2011;50(11-12):1207-13.

5. Pérez A, Sánchez T, Armengol N, Reyes F. Características y potencialidades de Moríinga *oleifera*, Lamark. Pastos y forrajes. 2010;33(4):1-16.

6. Colectivo de autores. Biocombustibles para su uso en motores Diesel. Capítulo 7, Potencial de la Moringa *oleífera* para la producción de biodiesel. La Habana, Gent: IDICT:2014:p.125-141.

7. Atabani AE, Silitona AS, Braduddina IA, Mahlia TMI, Masjuki HH, Mekhilef S. A comprehensive review on biodiesel as an alternative energy resourse and its characteristics. Renewable sustainable energy. 2012;16(4):2070-93.

8. Atabani AE, Braduddina IA, Mahlia TMI, Masjuki HH, Chong WT, Lee KT. Investigation of physical and chemical properties of potential edible and nonedible feedstocks for biodiesel production, a comparative analysis. Renewable sustainable energy. 2013;21:749-55.

9. Monfijur M, Masjuki HH, Kalam MA, Atabani AE, Arbab MI, Cheng SF, *et al*. Properties and uses of Moringa *oleifera* biodiesel and diesel blends in a multi-cylinder diesel engine. Energy Conversion and Management. 2014;82:169-76.

10. Mani S, Jaya S, Vadivambal R. Optimization of solvent extraction of Moringa *oleifera* seed kernel oil using response surface methodology. Food and Bioproducts Processing. 2007;85(C4):328-35.

11. Efeovbokhan VE, Hymore FK, Raji D, Sanni SE. Alternative solvents for Moringa *oleifera* seeds extraction. Journal of applied sciences. 2015;15(8):1073-82.

12. Martín C, Moure A, Martín G, Carrillo E, Domínguez H, Parajó JC. Fractional characterisation of jatropha, neem, moringa, trisperma, castor and candlenut seeds as potential feedstocks for biodiesel production in Cuba. Biomass and Bioenergy. 2010;34(4):533-538.

13. Marrero D, Vicente R, González VL, Gutiérrez J. Composición de ácidos grasos del aceite de las semillas de *Moringa oleífera* que crece en La Habana, Cuba. Revista Cubana de Plantas Medicinales. 2014;19(2):197-204.

14. NC 631:2008. Minerales-análisis granulométrico por tamizado-requisitos generales.

15. AOCS. Official methods and recommended practices. 5th ed, Champain, III. American Oil Chemists' Society. 1998.

16. AOCS. Official methods (Fatty acids composition). Ce 1b-89 for marine oils. 6th ed. American Oil Chemists' Society 2009.

17. Latif S, Anwar F. Quality assessment of Moringa *concanensis* seed oil extracted through solvent and aqueous-enzymatic techniques. Grasas y Aceites. 2008;59(1):69-75.

18. Anwar F, Rashid U. Physico – Chemical characteristics of Moringa *oleifera* seeds and seed oil from a wild provenance of Pakistan. Pakistan Journal of Botany. 2007;39(5):1443-53.

19. Thorton R and Neilson R. Organic Chemistry. Chapter 20, Functional Derivatives of Carboxylic Acids. Second edition. La Habana: Instituto Cubano del Libro: 1971:p.683-684.

20. Gomes SH. Extração enzimática de óleo e produção *in situ* de biodiesel a partir da Moringa *oleífera* Lam. [Tesis en opción del título de maestro en Ingeniería Química]. Ciudad de Natal, Universidade Federal do Rio Grande do Norte; agosto; 2013.