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Standardization of the oil extraction process of *Mauritia flexuosa* from Guaviare, Colombia

Estandarización del proceso de extracción de aceite de *Mauritia flexuosa* del Guaviare, Colombia

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Abstract *Maurita flexuosa* is a dioecious palm and its fruit is an oily drupe of which about 16.58 % of the mesocarp can be used for the process of transformation and extraction of oil, due to its high oleic acid index, of which its content of provitamins and polyunsaturated oils that contribute to its antioxidant activity. In order to determine the natural supply of *Mauritia flexuosa* in the municipality of San José del Guaviare, population studies were carried out using GIS and ArcGIS tools. The existing natural offer in the municipality corresponds to 1,351,175 palms in production and an estimated 140,977 tons of drupes ready for use. For the extraction of the oil, Soxhlet, Folch methods and cold pressing were evaluated, obtaining a maximum yield of 51 %, 13.35 % and 28.63 %, respectively. The experimental model called 2^K was used, with 16 trials where the effect of temperature, weight and pH was defined. Likewise, aspects of the market, costs, extraction processes and legislation were considered. Finally, the content of bioactives was verified by chromatography.

Keywords: Folch-method, cold pressed, Soxhlet method, oilseed.

Resumen *Maurita flexuosa* es una palma dioica y su fruto es una drupa oleaginosa de la cual cerca del 16.58 % del mesocarpio se puede aprovechar para el proceso de transformación y extracción del aceite, por su alto índice de ácido oleico, del cual se destaca su contenido de provitaminas y de aceites polinsaturados que contribuyen a su actividad antioxidante. Con el objetivo de determinar la oferta natural de *Mauritia flexuosa* en el municipio de San José del Guaviare se adelantaron estudios poblacionales con el uso de herramientas SIG y ArcGIS. La oferta natural existente en el municipio corresponde a 1.351.175 palmas en producción y un estimado de 140.977 toneladas de drupas listas para el aprovechamiento. Para la extracción del aceite se evaluaron los métodos Soxhlet, Folch y presado en frio, obteniendo un rendimiento máximo de 51 %, 13.35 % y 28.63 %, respectivamente. Se utilizó el modelo experimental denominado 2^K, con 16 ensayos donde se definió el efecto de la temperatura, peso y pH. Igualmente se tuvieron en cuenta aspectos del mercado, costos, procesos de extracción y legislación. Por último, se verificó el contenido de bioactivos mediante cromatografía.

Palabras clave: método Folch, prensado en frio, método Soxhlet, semilla oleaginosa.

Introducción

Mauritia flexuosa, commonly called Moriche, is a dioecious species, which can reach heights up to 40 m and have an average trunk diameter of 60 cm ending at the top with a crown between 15 to 20 leaves (Virapongse, *et al.* 2017). Females of *M. flexuosa* produce drupes in clusters of 25 kg including the raquila. This species is distributed in the Colombian Amazon and Orinoquia, forming a specific habitat commonly called morichal, low or canaguchal, mainly in floodable zones "old mothers" and on the slopes of the rivers, with a density of 27.5 individuals/ha, and in a ratio of 5 females/1 male (Endress, 2013).

An oil can be extracted from the palm fruits which is of commercial interest (Rull & Montoya, 2014) due to the presence of carotenoid antioxidants, tocopherols and monounsaturated fatty acids, with a higher content of palmitic, oleic and linoleic (Aquino *et al.*, 2012; Bailey & Shahidi, 2005) which possess bioactive substances such as provitamins (Cândido, Silva, Agostini & Costa, 2015) arousing interest at an industrial level for the production of cosmetic, food and animal food products.

The characteristics and composition of *M. flexuosa* oil have been researched (Milanez *et al.*, 2016; Milanez, Neves, Colombo, Shahab, & Roberto, 2018). Its main property is oxidant stability and secondly the content of tocopherols and pigments (Speranza *et al.*, 2016).

In relation to the extraction processes of essential oils with solvents, these consist in the removal of the crushed, laminated, milled, or previously pressed oil material, by washing with organic solvents in countercurrent (Mandal *et al.*, 2015). Usually after solvent extraction, the flour is carried to a toaster to recover the solvent. It is removed from the oil with film evaporators and vacuum distillation to subsequently extract other components such as dyes, gums, mucilages, waxes, fats, proteins, and carbohydrates (Sánchez, 2006).

The focus of the research is quantitative and explores the comparison of different essential oil extraction processes of *M. flexuosa* to determine if the process is environmentally, socially, and economically viable for the Amazon and Colombian Orinoquia. Likewise, the authors propose to generate a bioprospecting process, where a business unit can be located for different Family Agricultural Units (UAF) of the department of Guaviare.

Materials and Methods

Study area

The study area is located in the municipality of San José del Guaviare on the banks of the Guaviare River. The representative sample for the study was taken from an area of 38.605 ha, which correspond to 2.39 % of the 1.617.800 ha that the municipality has.

Figure 1 indicates the sampling area to carry out the collection of the natural supply information of the *M. flexuosa* species. During the sampling process, analyses of individuals were also carried out with the goal of obtaining a favorable usage percentage.

To determine the natural supply of M. flexuosa, population studies of the species were carried out in three representative areas of the region, on land plots of 100 x 40 m (4,000 m^2), evaluating the physical characteristics of each of the individuals present in the inventory. A peripheral polygon shape distribution of the zone and characterization of the forest cover of the low and flood areas, the characteristics of the drupe, harvesting and dewatering tests; determining the measurable parameters of each process.

Oil extraction is performed using the yellow mesocarp and processing it for the same purpose, i.e., approximately 16.6 % of the drupe is usable for oil extraction (Figure 2). The remaining material has various uses such as organic fertilizer, food products, among others.

Figure 1. Location of the Study Area.
Source: Google Maps. Adapted by the author.

Figure 2. Mesocarp of the drupe.
Source: SIEC (2018).

Extraction methods

Since there are numerous methods of oil extraction the determination of the extraction methods for the *M. flexuosa* oil was carried out considering the methodology analysis presented by Acosta (2019), the technological level of the department of Guaviare, the ease of appropriating the process and the human talent necessary to execute it.

Soxhlet extraction

Due to the structure of the Mauritia flexulosa pulp, petroleum ether is used due to its reaction, affinity, and polarity with the sample. For this type of extraction, dry pulp from the mesocarp and exocarp of the drupe were used. During drying, the water contained in the drupe was partially removed (75%). For the mixture, 20 g of dry pulp and 25 ml of solvent were used. After that, Soxhlet extraction is performed at a controlled temperature. The process takes two hours, and the separation of each component was done by means of broken evaporation.

Cold pressing extraction

In this method, 500 g of dry pulp were used, which were placed in the vertical press, exerting pressure directly on the mesocarp. The process is based on the breaking of vegetable fibers and oil drainage.

Folch Extraction

A mixture of chloroform and methanol in water was used at a ratio of 12/8 v/v. The dried pulp was homogenized with the solvent, leaving it then in incubation and stabilization. The organic and aqueous phase are separated, making the recovery of the organic phase and dissolved fat content possible.

Experimental design

The variables with which the experiment was developed (Table 1) are defined in input and output variables. Within the input variables, the temperature, pH, and weight of the drupe (minimum and maximum values) were considered. The output variables were the content of oil extracted, and the quantity and quality of its derivatives. In addition, factors such as human talent and resources were considered in order to establish an efficient development of the process and reduce costs.

Table 1

Experimental design

Factors	Level	Level	References
X1: Temperature (°C)	30	60	Extracting oil, 1910; Mandal *et al.*, 2015; Manhães *et al.*, 2015
X2: Weight (g)	41.18	56.16	36,48 g-78,07 g. Barbosa y Lima, (2010)
X3:pH ()	3.6	3.8	3,6-3,7 Resolución 2154 de 2012 (Minsalud)

Nota: Acosta (2019).

Data analysis is performed with STATISTICA v.10 software, to define the variability obtained between maximum and minimum ranges. In accordance with the parameters obtained in the bibliographic review, the experiment was set, and the replicates are defined. This software performs small variations among the variables, without considering the different types of extraction methods to which the raw material would be exposed (Table 2).

Table 2

Definition Experiments

Running schedule	Block	Temperature	Drupe Weight	pH	Can Oils
16	3	40,00	55,32	3,86	28,23
13	3	40,00	42,78	3,70	3,26
12	3	65,09	55,32	3,70	38,67
15	3	14,90	55,32	3,53	28,78
11	3	40,00	55,32	3,70	5,67
10 ©	2	14,90	55,32	3,70	28,53
2	1	40,00	56,16	3,80	10,34
9	2	30,00	56,16	3,80	49,38
4	1	60,00	56,16	3,60	51,46
17 ©	3	40,00	55,32	3,70	28,75
14	3	40,00	67,85	3,70	0
3	1	60,00	41,18	3,80	1,5
1	1	30,00	41,18	3,60	1,2
7	2	30,00	56,16	3,60	12,72
6	2	30,00	41,18	3,80	1,5

Note. Acosta (2019)

Considering the conditions expressed by the parameters defined for each run, the *M. flexuosa* pulp samples were organized in order to define the extraction method according to the temperature variable. Extractions with a temperature ≤30 °C were carried out with the Folch method, those with average temperatures up to 40 °C were carried out by pressing, and the extraction of oil with the Soxhlet method is carried out with a maximum temperature of 60 °C. The different tests (experiments) were established seeking compliance with the conditions determined by the software.

Results and discussion

In the species *M. flexuosa* the harvest of fruits is carried out in the plants According to the sampling carried out in the study area, it is possible to characterize the drupes as follows (Table 3).

Table 3

Description weight of the drupe

	Weight Milligrams	Standard deviation (mg)	Percentage weight
Drupe Weight	35.220	± 4820	100,00%
Exocarp	7.250	± 1270	20,58%
Yellow mesocarp	5.840	± 1580	16,58%
White mesocarp + almond	20.850	± 2520	59,20%
Losses during the process	1.280	± 340	3,63%

Note. Adapted from Acosta, G. et al. (2018).

For the modeling process, minimum and maximum values of the different variables were defined. For temperature, it is 30 °C minimum and 60 °C maximum, with a central value of 40 °C.

Bearing in mind that the bioactive ingredients of the oil vary due to the temperature used in the extraction process of *M. flexuosa* oil this implies, that the presence of carotenes can change as the temperature increases.

The weight of the fruits is directly proportional to their maturity index, which determines the physiological characteristics of the drupe such as weight, color, and size. The pH value defines the level of acidity or alkalinity of the fruit; on this variable, intervals defined by Hernández *et al.* (2018) are established.

mum and minimum values are established in STATISTICAL SOFTWARE and the input of the variables described above for the development of the experimental model is presented.

Table 4

Maximum and minimum values in Software STATISTICA. Software STATISTICA

Factor Name	Low Value	Low Label	Central Value	Low Center	High Value	High Label	Star Low Lobel	Star High Lobel
Temperature	30	Low	40	CenterPt	60	High	StarLow	StarHigh
Drupa Weight	41,18	Low	55,32	CenterPt	56,16	High	StarLow	StarHigh
pH	3,6	Low	3,7	CenterPt	3,8	High	StarLow	StarHigh

Note. Acosta (2019).

For the analysis of variance, STATISTICA software version 10 was used. In this, through mathematical calculations, the results of the experiments were analyzed and the interactions between temperature, weight and pH were compared. All this is defined under the null hypothesis denoted as: all groups of experiments come from the same population, in other words, all interactions have the same mean and variance.

The ANOVA (Table 5) is estimated with a significance level (p< 0,05). Considering the results of the model's different mathematical interactions, it can be inferred which factors are most relevant in the percentage of extraction of the Mauritia flexuosa oil. According with the statistical tool, the most relevant factors are weight of the drupe and temperature.

Table 5

Analysis of Variance (ANOVA)

Factor	ANOVA. Var: Cant. Aceite; R-sqr=,95158; Adj:,88933 (Spreadsheet1) 3 factors, 1 Blocks, 17 Runs; MS Residual=34,29276 DV: Cant Aceite.				
	SS	**DF**	**MS**	**F**	**P**
Temperature (L)	773,328	1	773,328	22,55075	0,002087
Temperature (Q)	2,089	1	2,089	0,06092	0,812132
Drupa weight (L)	2555,168	1	2555,168	74,51041	0,000056
Drupa weight (Q)	1365,211	1	1365,211	39,81046	0,000400
(3) pH (L)	1,235	1	1,235	0,03602	0,854860
pH (Q)	9,908	1	9,908	0,28894	0,607560
1L by 2L	527,893	1	**527,893**	15,39372	0,005723
1L by 3L	0,106	1	0,106	0,00308	0,957288
2L by 3L	1,181	1	1,181	0,03444	0,858035
Error	240,049	7	34,293		
Total SS	4958,002	16			

Note. SS = Sum of Squares. DF = Degrees of freedom MS= Root mean squared errors.

F= Test statistic (Estimated using probability distribution F). P= Probability.

Acosta (2019)

The incidence of each of the variables defined in the model is presented in figure 3 since the weight of the drupe is directly proportional to the level of maturity, it is advisable to control the *in situ* collection process of the moriche drupes, to obtain the greatest amount of oil. Additionally, temperature control defines the extraction method and is also a variable that directly impacts the presence of bioactive substances such as carotenoids.

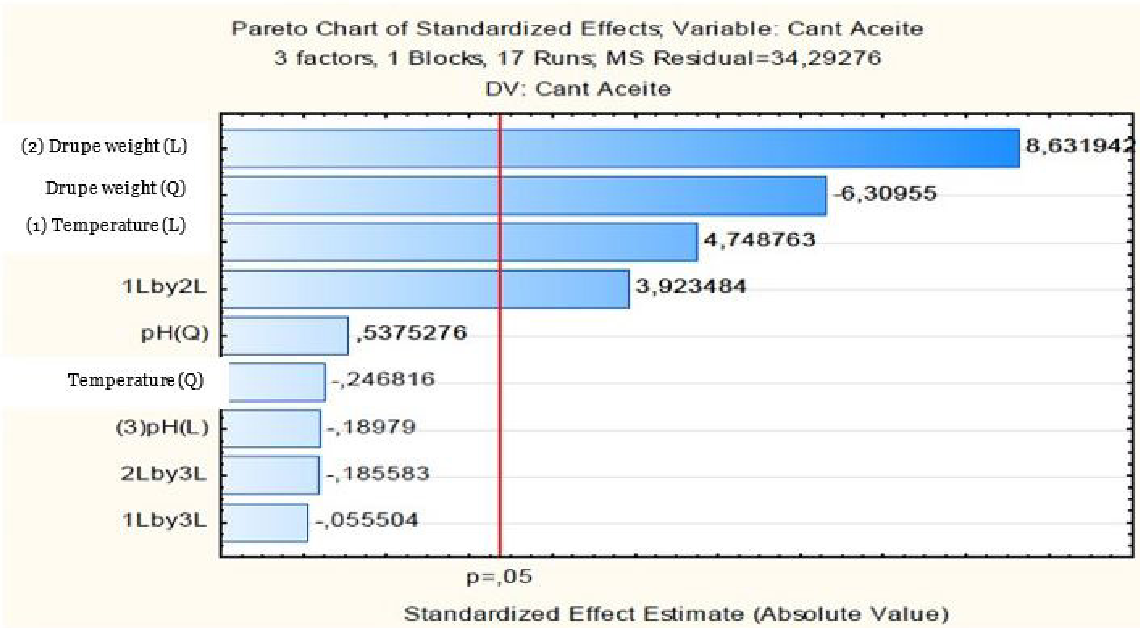

Figure 3. Surfaces..
Note. Acosta (2019).

In Figure 4, the interaction between the temperature and the weight of the drupe is presented, showing that the lower the weight, the lower the oil content. The preponderant factor is the immaturity of the drupe. It should also be noted that, according to the extraction method, the increase in temperature increases the extraction performance, which implies that if only the performance factor is analyzed, the Soxhlet method would be the first option, followed by cold pressing and finally the Folch method. Figure 4 delimits the different areas calorimetrically; the red colors show higher oil extraction rates, up to yellow with lower rates.

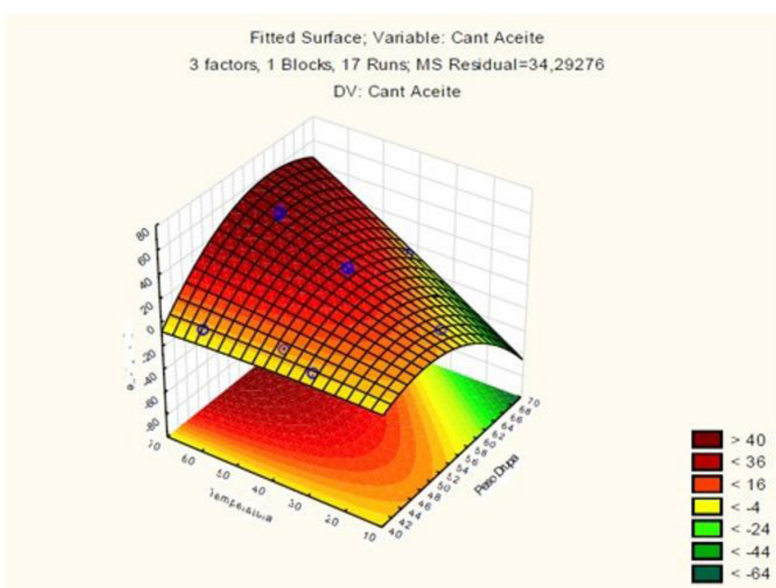

Figure 4. Colorimetry.
Source: Acosta (2019).

The best results were obtained with drupes between 54 and 56 g. Said interval defines the drupes in their mature state, and it also marks the extreme weights where drupes are observed between 40 and 44 g with extraction percentages well below the mature drupes.

Figure 5 projects the future behavior of the process, denoted by a correlation index R^2 = 0,95. In this way, it is possible to replicate the processes with a standard trend and with an acceptable level of error

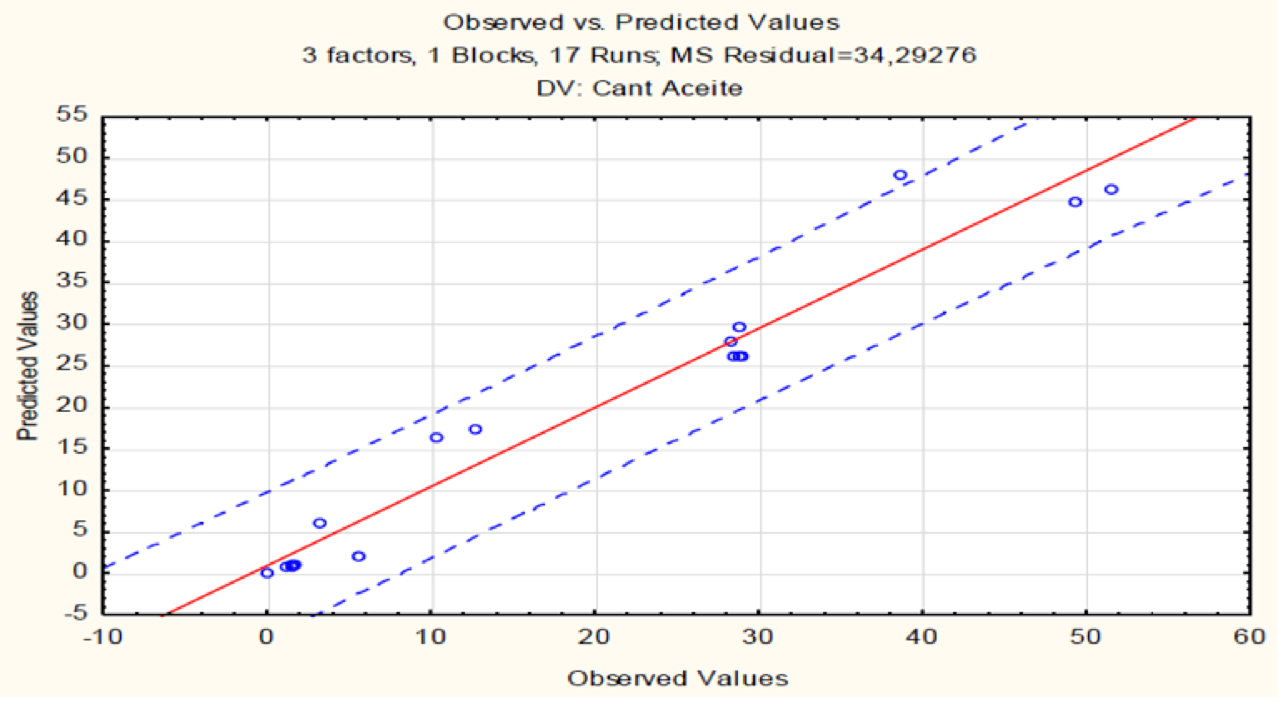

Figure 5. Correlation index.
Source. Acosta (2019).

After performing all the necessary tests to complete the statistical analysis described above, which shows the proportionality between drupe weight and temperature versus the percentage of oil extraction yield, it is concluded that the most relevant results were obtained using the Soxhlet extraction method with 51,4 %, while the Folch method achieved a maximum yield of 13,35 % (Table 6).

It is important to indicate that in the treatment of the sample for the case of fruits where only the mesocarp is removed, the Folch and Soxhlet extraction methods were applied. The pulp prepared for extractions with the Soxhlet and pressing methods is dehydrated beforehand.

Table 6

Oil extraction performance according to the method

Sample type	Amount	Method	Yield, (%)	Temperature Type °C
Ripe fruit	55,32 – 56,16 g	Folch maximum	13,35	30
Unripe fruit	41,01 ± 0,64 2 g	Folch minimum	6,44	30
	41,01 ± 0,64 2 g	Soxhlet minimum	1,5	60
Pulp (Unripe fruit)	41,01 g	Cold pressing minimum	1,2	40
Pulp	55,32 – 56,16 g	Soxhlet maximum	51,4	60
	55,32 – 56,16 g	Cold pressing minimum	28,63	40

Note. Acosta (2019).

The laboratory samples were developed by verifying the ripeness status of the fruit (Table 7).

The characteristics of the oil were verified according to the extraction method, not including Folch, due to the poor performance reported in the tests; in this procedure, the density, refractive index, acidity of the oil, peroxide index and iodine index are determined. All these variables are quality considerations of the oil extracted (Table 8). The density of the oil defines the content of fatty substances that are suspended within the oil. The refractive index determines the purity of the oil, the acidity and iodine index of the extracted samples specifies the content of fatty acids present in the sample and the peroxide index defines the level of oxygenation that the oil presents.

Table 7

States of maturity

Ithem	State of madurity	Sample
T1	Unripe fruit	41,01 ± 0,64 2 g
T2	Pulp	55,32 – 56,16 g
T3	Ripe fruit pulp	55,32 – 56,16 g

Note: Own elaboration

Table 8

Characteristics of M. flexuosa oil and quality parameters

Muestra	Método	Densidad (g/ml)	Índice de refracción	Acidez (meq de koh/g)	Índice de peróxidos (meq de peróxidos/Kg)	Índice de yodo (g de I/100 g de muestra)
Moric pulp treatment 1 (t1)	Soxhlet	0,854	1,467	1,759	8,742	71,163
	Cold pressed	0,859	1,4671	3,78	6,37	78,255
Moric pulp treatment 2 (t2)	Soxhlet	0,858	1,4657	3,23	13,034	74,023
	Cold pressed	0,855	1,467	5,779	9,181	86,656
Moric pulp treatment 3 (t3)	Soxhlet	0.855	1,4565	4,421	18,032	94,111
	Cold pressed	0,852	1,4565	6,155	12,217	90,774
Moriche oil	Cold pressed				6,779	

Note: Own elaboration.

According to the results of the quality conditions of the extraction by the Soxhlet and cold pressing methods, the compliance rates were verified against the standard, and specifically to Resolution 2154/2014 of the Ministry of Health of the Republic of Colombia.

Table 9

Aspects of Mauritian flexible oil market.

Extraction Type	Market	Costs	Extraction Process	Legislation
Folch	Due to the presence of several solvents in the oil, according to regulations, the use for cosmetic or food products is not possible	$140.000/30 g of pulp. Expensive solvents. Equipment High cost	The process is complex. Logistics special warehousing conditions. The performance of *M. flexuosa* oil extraction is low.	Resolution 2154 of 2012 Determines the conditions as bleached or discolored oils. Lower price on the market
Soxhlet	Using the solvent petroleum ether, it can be used for the cosmetics and food industry, carrying out studies where it is demonstrated that the oil does not end with traces of solvent. The oil that is extracted is called natural crude	$72.000/10 g of flour. Reusable solvent. Equipment High cost	The process is simple. Logistics (special conditions of solvent storage). The performance of *M. flexuosa* oil extraction is low.	Resolution 2154 of 2012 determines the conditions as natural crude oil. Average market price
Cold pressed	The market gives more value to virgin oils and fats	$150.000/1000 g of flour. No solvents. Average cost equipment	Simple process. Solvent – free logistics process. Oil extraction has an average performance	Resolution 2154 of 2012 determines conditions for virgin oils and fats. Higher price on the market

Note. Acosta (2019)

The results show that the Soxhlet method exhibits outstanding performance with 51,4 % oil. Despite this, it is important to consider other variables that influence the choice of the type of extraction to be used, such as: market value, costs, extraction processes and legislation.

In terms of market value, to generate a productive unit it is necessary to take the product with the highest possible value (Table 9).

The structuring of the production plant (Figure 6) is established considering the aspects presented in Table 9. The cold pressing method is the most recommended for the extraction of *M. flexuosa* oil, because although it does not generate the highest yield, it is the method that least compromises the bioactive existing within the molecular structure of the oil.

The initial process is described with theoretical capabilities, it begins with the pulping unit of the drupe with a capacity of 100 to 300 kg per hour. The machine is fed with the dry mesocarp, the by-products derived from the pulping amount to 84 % of the drupe, which is the endocarp and exocarp. The dehydrator has a capacity of 5 kg of pulp per tray; the process unit is made by a solar dryer model and can last from 12 to 18 sunshine hours. The milling of the flour obtained from the dehydration process unit has a theoretical capacity of between 75 to 300 kg per hour with a loss of 1 %.

The cold press takes between 2 hours and 47 minutes to process 170 kg of flour, and it is filtered after the cold pressing without altering the physicochemical properties of the oil, in which it has a loss of 5 % and a capacity of 60 to 400 L/h. When filtering 54 kg, due to the oil density that is 0,91, it results in approximately 50 L of extra-virgin olive oil per day. Finally, the oil enters the filter press to extract impurities derived from the extraction process.

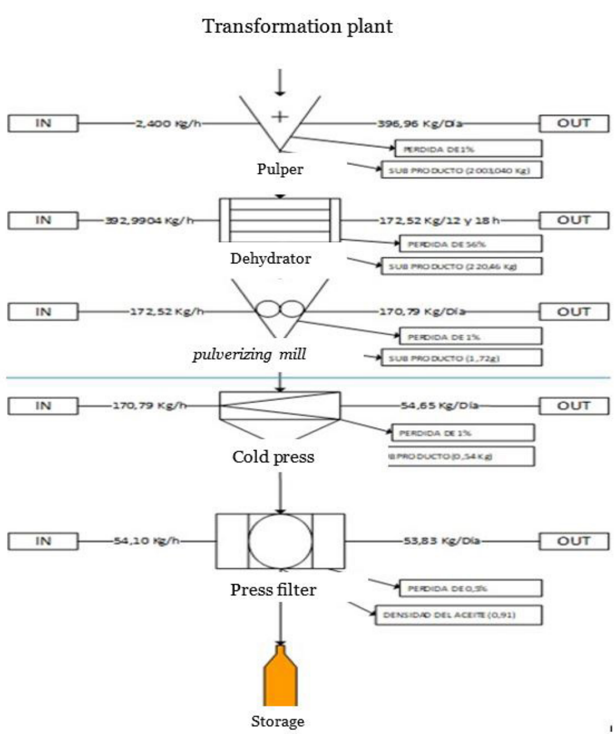

Figure 6. Processing plant.
Note. Acota, G. *et al*. (2018).

According to studies carried out by Araújo, V. F. *et al.* (2007) and Araújo, M. E. *et al.* (2000) when evaluating the oil quality variable for both crude and refined oil, based on five quality conditions; it is evident that in two of them (peroxide index and iodine index) the results obtained for the extracted crude oil comply with the quality parameters. For the three remaining conditions (density, refractive index, and acidity index) the variability according to the theoretical references is due to environmental conditions.

Conclusions

The department of Guaviare has a natural *M. flexuosa* offer that can be used to generate productive units as alternatives to Family Agricultural Units in the four municipalities of the department in a sustainable way.

For the generation of a standardized production unit for the extraction of *M. flexuosa* oil, it is important to improve the unitary operation of the drupe collection, to reduce the environmental impacts generated by said extractive activity.

The cold pressed extraction method, with a maximum yield of 28,63 %, has the same quality conditions as the oil extracted by the Soxhlet method; but according to the resolution of the Ministry of Health 2154/2014, the classification of the oil under the cold-pressing process is classified as a crude oil, which has a higher value at the commercial level than the refined oil obtained by the Soxhlet extraction method.

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