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Articulo Original

Cultivatión of *Moringa Olifera Lam.* and its manufacture for human consumption as a nutraceutical

Cultivo de *Moringa oleifera Lam*. y su manufactura para consumo humano como nutracéutico

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Abstract

Objective: To determine the ideal planting density for a higher foliar yield of *M. oleifera* with the presence of compounds that contribute to biological effects of interest for human health. ***Methodology:*** Leaves were harvested 45 days after planting. Yields were quantified, and the industrial leaf processing and the bromatological characterization were evaluated. ***Results:*** A 42000 plants ha^{-1} planting density reached the highest yield of fresh and dry leaves, with 7.16 and 0.89 t ha^{-1}, respectively. The antioxidant, anti-inflammatory and hypolipidemic effects of the dried green and yellow leaves, the presence of isoquercitrin as an active compound and its use in food fortification were demonstrated. ***Conclusion:*** Under the climatic conditions in Havana, Cuba, a high foliar yield of *M. oleifera* can be obtained, which after drying retained its excellent bromatological characteristics favoring its use in food fortification. It has an antioxidant, anti-inflammatory, hypolipidemic, anti-diabetic and non-mutagenic effect; the yellow leaves can also be used as a nutraceutical fortifier.

Keywords: planting distance, phenols, flavonoids, bromatology.

Resumen

Objetivo: Determinar la densidad de siembra idónea para un mayor rendimiento foliar de *M. oleifera* con la presencia de compuestos que tienen efectos biológicos de interés para la salud humana. ***Metodología:*** Las hojas se cosecharon 45 días después de establecidas las plantaciones. Se cuantificaron los rendimientos, se evaluó el procesamiento industrial de las hojuelas y la caracterización bromatológica. ***Resultados:*** La densidad de siembra de 42 000 plantas ha^{-1} obtuvo el mayor rendimiento de hojas frescas y hojuelas secas, con 7,16 y 0,89 t ha^{-1}, respectivamente. Los resultados de los metabolitos fueron elevados, no influenciados por el incremento de la densidad de siembra. Se demostraron los efectos antioxidantes, antiinflamatorios e hipolipidémicos de las hojuelas secas verdes y amarillas, la presencia de isoquercitina como compuesto activo y su uso en la fortificación de alimentos. ***Conclusión:*** Bajo las condiciones climáticas en La Habana, Cuba, se puede obtener elevado rendimiento foliar de *M. oleifera*, que después de seco, conservó sus excelentes características bromatológicas, que favorece su uso en la fortificación de alimentos; las hojuelas amarillas pueden también ser aprovechadas como nutracéutico.

Palabras clave: distancia de siembra, fenoles, flavonoides, bromatología.

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Introduction

The presence and utilization in Cuba of *Moringa oleifera* dates back two centuries. It is naturalized and distributed all over the country. It is a plant native to the Himalayas (Kou *et al*., 2018), growing currently in tropical and subtropical regions of the planet; it is easy to propagate, both from seeds and vegetative material with great ecological plasticity, since it adapts to a wide range of soils (Benítez *et al*., 2018; Hernández & Hernández, 2018; Álvarez, 2019)and withstands drought (Fejér *et al.*, 2019).

Moringa plants grow fast, which makes them easier to harvest when grown to produce fresh dehydrated biomass, so the harvest period could be from 45 to 60 days (Chepote, 2018). This plant shows a wide range of benefits, so it is considered one of the most useful trees (Gopalakrishnan *et al.,* 2016). It plays an important role in the technological and scientific development of interest to man, due to the vast prospect of possible novel biomedical, nutritional agricultural and industrial applications in food science (Khan, 2018; Aderinola *et al*., 2020). The introduction and generalisation of agricultural technologies to promote the crop for consumption with proven efficacy to reach high leaf production is an essential objective to meet the increasing demand for final products for the Research Center on Protein Plants and Bionatural Products, responsible for this scientific result. *Moringa oleifera* has been well-recognized as a phytonutrient-rich vegetable-producing plant.

There has been increasing interest in consuming exogenous natural-derived antioxidants in recent years. Several antioxidants isolated from plant secondary metabolites, particularly polyphenols, such as phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes are positively related to protective effects against age-related diseases and chronic metabolic disorders, which are the leading global public health challenges (Vonghirundecha, 2022). That is why the interest of the present study was to determine the optimal planting density to get high foliar yields of *M. oleifera* with the presence of compounds that contribute to biological effects of interest for human health.

Methodology

Agricultural performance and yield evaluation

Plant material botanically identified as *Moringa oleifera* Lam. 1783, ecotype Nicaragua, was used. Planting was done in May 2020 with 40 days old seedlings, geographically located at 23° 04' 20" N and 82° 29' 20" E. Four planting distances (planting density) were used: 1 m x 1 m (10 00 plants ha^{-1}), 1 m x 0.5 m (20000 plants ha^{-1}), 0.6 m x (double furrow) 0.4 m x 0.6 m (33000 plants ha^{-1}) and 0.8 m x 0.3 m (42000 plants ha^{-1}). The agricultural and industrial yields of dry flakes and bromatological properties were determined (Oficina Nacional de Normalización, 2020; Oficina Nacional de Normalización, 2022).

Antioxidant, anti-inflammatory and hypolipidemic effects (activity)

A phytochemical screening was done to determine the total polyphenols, tannins, flavonoid content and antioxidant activity (Lago et al., 2021). To evaluate the anti-inflammatory effect, male mice OF-1 were used with the implanting models of granuloma induced by cotton speck and sole oedema by the administration of carrageenan (Lago et al.*,* 2021). To determine the hypolipidemic effect, Swiss albino male mice were used, as per the model induced by Tyloxapol. The effect of powder capsules from dry leaves at doses for one and eight days was also evaluated and biochemical parameters were determined as previously described (Lago et al.*,* 2021). At the end of the experiment, the animals used were sacrificed under anaesthesia.

Identification and quantification of isoquercitrin

The presence of isoquercitrin was identified by thin-layer chromatography (TLC) in pre-elaborated plica gel 60 F 254. The plates were stained with 5% (v/v) $FeCl_3$ in 5% ethanol as a chromogenic agent and incubated at 105 °C for 5 minutes. For the detection of the corresponding bands, they were visualised with UV light at 254 and 365 nm. (Almora-Henández *et al*., 2021). The quantification through high-pressure liquid chromatography (HPLC). A Shimadzu system was used under the following working conditions: mobile phase: acetonitrile: H_3PO_4 in water 0,1% (15:85); column: Supelco ascentis C18 (25 cm x 4.6 mm x 5 µm); detector: diode array (DAD); pump LC-20AD, readings at 255 nm; flow: 1 mL min^{-1}; injection volume: 20 µL and standard: isoquercitrin (Merck). (Lago Abascal, Almora Hernández, González García, Campa Huergo *et al*., 2021).

Toxicity and mutagenic effect of Moringa flakes

In the evaluation of toxicity, non-pregnant female mice Cenp/Wistar of corporal weight from 150 and 200 g were used. The mutagenic effect evaluated the genotoxic activity through the system of Bacterial Reversion Essay in *Salmonella typhimurium* (Salmonella/Microsoma) isolates TA 98 and TA 100. Both services were contracted with the National Research Center for Medicines (CIDEM), in Havana, Cuba.

The use of moringa in food fortification

Integral rice cookies with water extract from leaves of *M. oleifera* and *Stevia rebaudiana* were evaluated. Physical and chemical variables of the cookies, sensorial analysis and performance of quality parameters were determined (Almora *et al*., 2020; Almora *et al.,* 2021; Lago *et al.,* 2021).

Characterization of yellow leaves

For green and yellow leaves of *M. oleifera*, the total extractable solids (TES), chlorophylls *a* and *b*, total anthocyanin, polyphenols, flavonoids and macronutrients were determined (Lago *et al.,* 2021).

Statistical analysis of the samples

The software SPSS Statistic 23, IBM, was used for statistical processing when necessary.

Result and discussion

Agricultural performance and yield evaluation

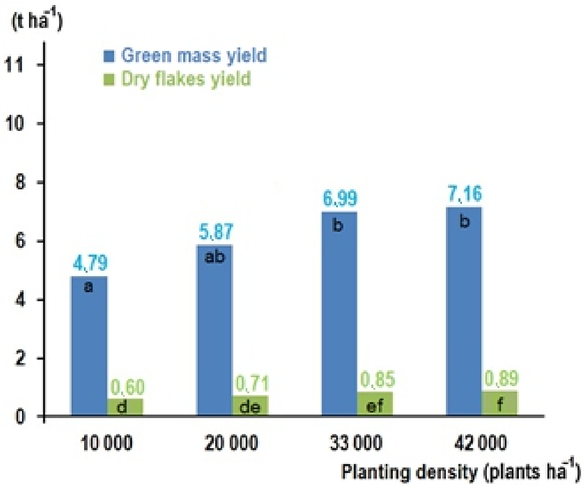
The average plant height value 42 days after planting was similar in spacings 1 m x 1 m (10 000 plants ha^{-1}) and 1 m x 0.5 m (20000 plants ha^{-1}), with 154.1 and 152.7 cm, respectively, the same as the average diameter of stems, with 2.3 cm in both cases. In plots 0.6 m x (double furrow) 0.4 m x 0.6 m (33000 plants ha^{-1}) and 0.8 m x 0.3 m (42000 plants ha^{-1}), plant growth reached 117.5 and 129.1 m, respectively, while stem diameter was 1.8 cm in both cases. Growth was faster and similar in planting densities 1 m x 1 m (10000 plants ha^{-1}) and 1 m x 0.5 m (20000 plants ha^{-1}), with significant differences regarding plots 0.6 m x (double furrow) 0.4 m x 0.6 m (33000 plants ha^{-1}) and 0.8 m x 0.3 m (42000 plants ha^{-1}).

Fresh flakes and rachis yield per crop area showed an ascending growth that tended to increase with the evaluated planting densities, 2.62; 3.21; 3.9 and 4.05 t ha^{-1}, respectively. Apical bud yield behaves differently; it reached the highest value for planting densities 1 m x 0.5 m (20000 plants ha^{-1}) and 0.6 m x (double furrow) 0.4 m x 0.6 m (33000 plants ha^{-1}), with 0.8 t ha^{-1}, without significant differences regarding planting density 0.8 m x 0.3 m (42000 plants ha^{-1}), where it was 0.76 t ha^{-1}, whereas density 1 m x 1 m (10000 plants ha^{-1}) reached 0.43 t ha^{-1}. On the other hand, the yield of stems left in the field, about non-used plants in manufacturing dry leaves for nutraceutical use, showed significant differences in all cases, with a downfall behavior that tended to increase with increasing planting density from 1 m x 1 m (10000 plants ha^{-1}) up to 1 m x 0.5 m (20000 plants ha^{-1}), equal to 10.75 and 10.38 t ha^{-1}, respectively. However, plots 0,6 m x (double furrow) 0.4 m x 0.6 m (33000 plants ha^{-1}) and 0.8 m x 0.3 m (42000 plants ha^{-1}), showed that the quantity of stems left in the field tended to increase with 10.82 and 11.82 t ha^{-1}.

Yield result of plant matter making up the manufacturing process of dry flakes as nutraceuticals and its drying is shown in Figure 1. Yield, both of leaves and dry flakes, increased compared to increased densities from 1 m x 1 m (10000 plants ha^{-1}) to 0.8 m x 0.3 m (42000 plants ha^{-1}). It is been mentioned that high foliar area indexes increased the interception of photosynthetically active radiation (Tinoco *et al*., 2008) and the accumulation of dry matter in crops (Cuvertino, 2020).

Figure 1

Yield performance of the industrial processing according to different planting densities. ANOVA gl: 3, 32; leaves yield, F 10.438; p = 0.000 and dry flake yield, F 10.796; p = 0.000; according to Scheffe test: Unequal letters mean difference for p < 0.05.



The accumulation of pigment in flakes relative to density, was similar for β-carotenes, carotenoids and anthocyanins, with a tendency to increase from 1 m x 1 m (10 000 plants ha^{-1}) till 0.6 m x (double furrow) 0.4 m x 0.6 m (33000 plants ha^{-1}) (table 1). The total chlorophyll concentration decreased as planting density increased. For planting density 0.8 m x 0.3 m (42000 plants ha^{-1}), the presence of flakes of β-carotenes, carotenoids and anthocyanins slightly reduced.

Table 1

Pigment in flakes according to planting densities

Planting densities	β-carotenes (mg mL^{-1})	Carotenoids (μg mL^{-1})	Anthocyanins (g L^{-1})	Total chlorophyll (μg mL^{-1})
1 m x 1 m (10000 plants ha^{-1})	0.98	5.94	0.22	12.11
1 m x 0.5 m (20000 plants ha^{-1})	1.08	6.53	0.24	11.02
0.6 m x (double furrow) 0.4 m x 0.6 m (33000 plants ha^{-1})	1.24	7.50	0.28	10.41
0.8 m x 0,3 m (42000 plants ha^{-1})	1.17	7.08	0.26	11.24

The analytical processing showed no effect of the density over pigments in dry flakes, total chlorophyll, β-carotenes, carotenoids and anthocyanins ($p > 0.05$). The values of carotenoids and β-carotenes were lower than 1,93 and 0,93 mg, respectively, reached in other studies on dry flakes of moringa (Nambiar & Seshadri, 2001).

Table 2

Bromatological analysis in leaves according to planting densities.

Planting densities	Moisture (%)	Protein (%)	Ashes (%)	Fats (%)	Starch (%)
1 m x 1 m (10000 plants ha^{-1})	8.9	32.3	11.3	10.95	0.6
1 m x 0.5 m (20000 plants ha^{-1})	8.7	31.7	11.0	12.2	0.6
0.6 m x (double furrow) 0,4 m x 0.6 m (33000 plants ha^{-1})	8.8	33.1	11.2	12.1	0.6
0.8 m x 0.3 m (42000 plants ha^{-1})	8.8	31.1	11.2	12.5	1.2

The bromatological analysis was similar for planting densities as to variables like moisture. protein and ash contents (table 2). Regarding moisture, proteins, fibers and ashes (table 2), the highest percentage was reached with the planting density of 1 m x 1 m (10000 plants ha^{-1}), whereas for fats and starch, it was reached with the highest planting density. In general, metabolite results were high, but not influenced by increased planting density.

Antioxidant, anti-inflammatory and hypolipidemic effects (activity)

In the current study, polyphenols content (table 3) was found to be higher for planting density 1 m x 0.5 m (20000 plants ha^{-1}), with 185.87 mg equivalent Gallic Acid g^{-1} dry base (mg EAG g^{-1} s.b), compared to another evaluated planting densities. Whereas, a different performance was recorded for flavonoids, where the highest value was for planting density 0.6 m x (double furrow) 0.4 m x 0.6 m (33000 plants ha^{-1}), which reached 170.51 mg equivalent of quercitrin per gram dry base (mg EQ g^{-1} s.b).

Table 3

Secondary metabolites in leaves according to planting densities: polyphenols and flavonoids.

Planting densities	Polyphenols (mg EAG g^{-1} s.b)	Flavonoids (mg EQ g^{-1} s.b)
1 m x 1 m (10000 plants ha^{-1})	72.81±8.63	65.38±4.10
1 m x 0.5 m (20000 plants ha^{-1})	185.87±60.39	139.96±80.74
0.6 m x (double furrow) 0.4 m x 0.6 m (33000 plants ha^{-1})	154.72±34.18	170.51±34.60
0.8 m x 0.3 m (42000 plants ha^{-1})	98.50±4.76	82.55±2.43

The oral administration of the dry flake extract in the model of induced granuloma significantly reduced the weight of the granuloma. The rates of 50, 100 and 150 mg kg^{-1} caused a percentage reduction of this parameter that reached 42.1; 52.9 and 42.9%, respectively. The reduction attained for the highest tested rate (300 mg kg^{-1}) was 18% lower than that recorded for lower rates without reaching significant levels compared to the control group in the study.

The evaluation of the feet edema model induced by carrageenan showed a significant reduction of the edema, both in its early and late stages. The oral administration of Moringa caused anti-inflammatory effects, both for the chronic and acute inflammatory models, which indicate its simultaneous influence on different events involved in the inflammatory process with a shaped "U" response.

It was evident that the treatment with a Moringa capsule had a better effect on total cholesterol than on triglycerides, with 341.40±26.11 and 814.0±75.34 mg dL^{-1}, respectively.

With the continued treatment of two capsules, the response was better regarding low-density cholesterol (LDL), with 20.60±1.51 mg dL^{-1} and an increase in high-density cholesterol (HDL) equal to 39.02±4.81 mg dL^{-1}, compared to the results with a dose of 18.20±1.40 and 39,94±3,34 mg dL^{-1}, respectively. These results demonstrated that Moringa flakes have compounds with hypolipidemic properties.

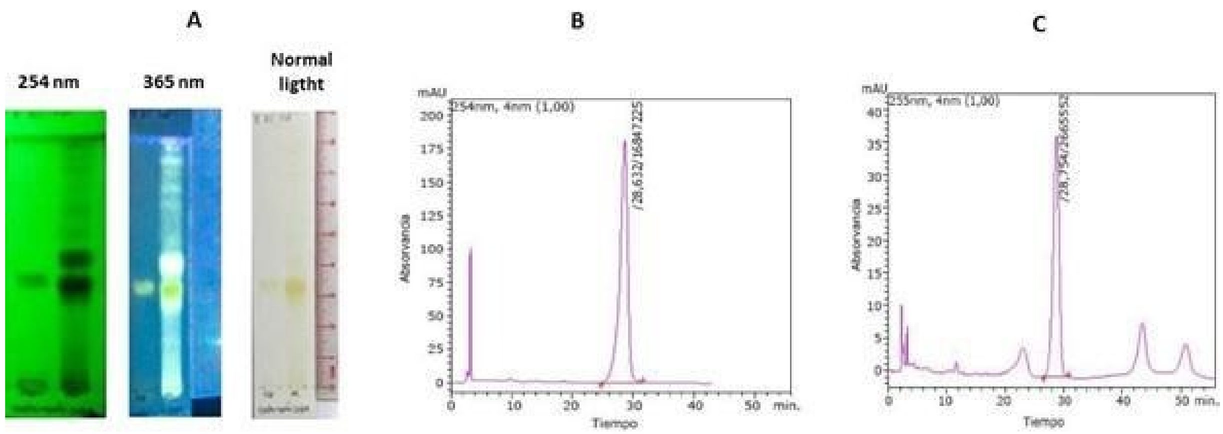
Identification and quantification of isoquercitrin

The thin layer chromatography (Figure 2) showed some bands of isoquercitrin at the wavelengths 254 and 365 nm, with an Rf value equal to 3.4. The quantitative analysis by HPLC showed the existence of a majority peak at 28 minutes, similar to the isoquercitrin pattern. The isoquercitrin content in the ethanol extract of dry Moringa flakes was 0,128%.

The presence of isoquercitrin suggested this metabolite is one of the responsible ones for the observed biological effects. The antidiabetic and antimicrobial effects of the plant were also shown as it was previously described (Lago *et al*., 2020; Hernández & Iglesias, 2021).

Figure 2

Presence of isoquercitrin in Moringa oleifera flakes: A- Analysis by thin layer chromatography, B- Analysis by HPLC of control isoquercitrin, C- Analysis by HPLC of flakes.



Toxicity and mutagenic effect of Moringa leaves

The autopsy did not reveal pathological disorders by the macroscopic analysis of the organs in the groups under study. Results proved that after the administration of flake powder, no toxicity symptoms at the rate of 1.334 mg kg^{-1}, showed up; the median lethal dose (DL_{50}) could be determined in the range of 2000 and 5000 mg kg^{-1}. A response bound to the concentration was not evident, either.

The use of Moringa in food fortification

Moringa cookies supplemented with 10% and 20%, reached significantly higher values as to protein content (between 7.56 and 9.1%) and fats (between 2.75 and 3.3%), compared to the basic integral rice cookie that had 6.92 and

2.80%, respectively. Fibre showed similar values in all the groups of Moringa-supplemented cookies compared to the basic cookie. Such values were according to literature references of 7.48 % (Abdalla, 2013). Ash content behaved similarly in all groups, as well as the starch, which showed values from 54.05 to 61.38%, indicative of the energy contribution of this product. Sensorial tests showed the addition of Moringa at 10 and 20% did not affect the attributes of integral rice cookies and significantly boosted their nutritional value. Cookies supplemented with Stevia at 5 and 10% reached the highest acceptance, according to the hedonic survey. They fell in the category "I Like" with 37.2 and 57% of acceptance, respectively. The group (10% Stevia), by having a sweet flavour, reached 81% of acceptance, higher than the group (5% Stevia), which was only slightly sweet.

Characterization of yellow leaves

The bromatological analysis showed differences between yellow and green flakes. Chlorophyll *b* in yellow flakes was lower with 3.15±0.04 µl mL^{-1} than chlorophyll *a* with 5.93±0.03 µl mL^{-1}. The β-carotenes content was lower in yellow flakes compared to green ones, where it reduced from 39.87±0.05 to 0.43±0.03 (mg 100 mL^{-1}). The proximal composition showed a higher value of starch in green flakes at 14.81% and ashes at 13.56%, compared to green ones; whereas fats and proteins reduced to 8.74 and 20.76%, respectively. Flavonoids content was higher in green flakes with 110.07±0.06 mg EQ g^{-1} s.b compared to yellow ones with 69.43±0.03 mg EQ g^{-1} s.b, whereas the polyphenols content was higher in yellow flakes with 289.01±0.01 mg EAG g^{-1} s.b than in the green ones 93.03±0.06 mg EAG g^{-1} s.b. In general, metabolites were high and not influenced by an increased density. Results showed that yellow leaves could be used in food products similarly to green ones, mainly when the green colour affects the sensorial acceptance of the fortified food. This research provides information on the nutritional value of this plant; it is recommended to use yellow leaves in the food industry, though for a similar fortification at a higher proportion than green leaves.

Conclusion

Under the climatic conditions in Havana, Cuba, a high foliar yield of *M. oleifera* can be obtained, which after drying retained its excellent bromatological characteristics favoring its use in food fortification. It has an antioxidant, anti-inflammatory, hypolipidemic, anti-diabetic and non-mutagenic effect. The yellow flakes can also be used as a nutraceutical fortifier.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author´s contributions

Conceptualization: Efraín Rodríguez; Investigation: Ernesto Almora, Vivian Lago, Raisa Monteagudo, Susana Matos, Efraín Rodríguez; Methodology: Ernesto Almora, Vivian Lago, Raisa Monteagudo, Efraín Rodríguez; Supervision: All authors; Writing - original draft: Ernesto Almora, Susana Matos, Efraín Rodríguez; Writing - review and editing: Ernesto Almora, Susana Matos, Efraín Rodríguez.

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