EVALUATION OF ENZYMATIC ALTERNATIVES FOR THE EXTRACTION OF MANGO SEED OIL (Mangifera indica Linnaeus.)

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ABSTRACT

The high production of mango seeds (Mangifera indica linnaeus.) in the Dominican Republic, and especially those from processing companies of this fruit, represents an opportunity to find new possibilities that lead to the use of these wastes in different scenarios. The oils are mainly used for human consumption, animal feed, soaps, cosmetics, biodiesel production and hybrid vehicle motor fuels. In accordance with the above, it is necessary to provide innovative proposals aimed at finding new sources of vegetable oil that allow providing new alternatives in the production of this resource. This new alternative of the use of mango would be an alternative for the use of by-products from the food industry and thus contribute to the reduction of possible environmental impacts. In this investigation it was concluded that the percentage of oil extraction using enzymes was low compared to the extraction by hexane. The mango seed was hydrolyzed using a ternary combination of 65% Alcalase, 25% Viscozyme L and 10% Neutrase. The enzymatic methodology presents an alternative for the extraction of mango seed oil with cosmetic and food applications.

Keywords: Mango butter, mango seeds, mango peel, processing of mango.

1. INTRODUCTION

The mango (Mangifera indica L.) originates from India and Southeast Asia where more than a thousand varieties have been cultivated. At the Latin American level, Brazil was the first country where the mango was cultivated, from here it was taken to Barbados in 1742 and to Jamaica in 1872. By the year 1800 it was already known in Central America and at that time it was taken from Cuba to Florida. Southeastern US, where the commercial varieties grown today were developed. The fruit is a drupe that can contain one or more embryos. Indian-type mangoes are monoembryonic and most commercial cultivars are derived from them. Generally, polyembryonic shanks are used as patterns. The mango has been distributed throughout Southeast Asia and the Malay Archipelago since ancient times. India probably has more commercial plantations than the rest of the world combined. However, the real economic importance of the mango consists of its high level of local consumption that takes place in every town and city in the lowlands of the tropics, since it is one of the most fruitful plants in tropical countries (Gastón, 2011).

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Mango (Mangifera indica L.) is consumed worldwide for its sensory delights and nutrient package, but its nutrient-rich by-products such as peel and seed are wasted by food industries. These by-products have received the attention of researchers for their great functional and nutritional potential. Extracted mango butter has been extensively studied for its potential use in both the food and non-food industries. However, more research is still required to discover efficient extraction methods and discover more applications of mango butter (Qureshi, Nayak, Kim, & Pal, 2018).

Mango production (*Mangifera indica* L.) has become an important economic activity in our country, due to the development it has had in recent years and the foreign exchange generated by exports. In 2018, the Dominican Republic received foreign currency in the order of US$16.48 million for mango exports, the main producing areas being Azua, San Cristóbal, Baní, Neiba, San Juan, Barahona, Pedernales, Dajabón, El Seibo, La Altagracia, among other provinces. The main mango varieties grown in the country in commercial plantations: Introduced varieties: Keitt, Kent, Tommy Atkins, Palmer. Creole cultivars are: Mingolo, Gold Cream, Banilejo, Gold Drop. Production tasks have increased 14 thousand tasks since 2017, currently the country has an estimated planting area of 120,000 tasks, these plantations have an average yield of 11 tons/ha (Agriculture, 2021).

Currently, the primary production and industrialization of fruits generates by-products in large quantities, which cause pollution that cause environmental problems, in addition to economic losses, since they are not used efficiently. During the processing of mango (Mangifera indica L.), for example, in the production of juices, only small portions of it are used and considerable amounts of peel and seeds are discarded as industrial waste (García, López, Saucedo, Salazar, & Suárez, 2015), which are estimated to be around 75,000 t, worldwide (Dorta, Lobo, & González, 2012). It has been found that the peel constitutes 15-20% and the seed between 35-60% of the total weight of the mango (Jahurul, Zaidul, Ghafoor, Al-Juhaimi, & Nyam, 2015).

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Mango butter (*Mangifera indica* L.) is one of several exotic fats that were evaluated beginning in the 1930s as alternatives to cocoa butter in confectionery products. Subsequent research shows that its appreciable content of tocopherol, phytosterols and triterpenes make mango butter a functional cosmetic ingredient with great potential as a natural complement in cosmetic formulations (Hernández, Aldana & Montoya, 2016).

Its use in the cosmetic industry allows us to offer a versatile product, when used in cosmetic products it provides a protective and regenerative effect on the skin, it has vitamins (E, A, B1, B2, B3), fatty acids, proteins, minerals that make of this oil, the best of its kind with a real contribution to people's well-being.

Enzymes are very powerful and effective catalysts, chemically they are proteins. As catalysts, they act in small quantities and are recovered indefinitely. They do not carry out reactions that are energetically unfavorable, they do not modify the direction of chemical equilibria, but rather accelerate their achievement. The most outstanding characteristic of enzymes is their high specificity.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Location of the Study: In the research laboratories of the Department of Pharmaceutical Biotechnology of the Institute for Innovation in Biotechnology and Industry (IIBI), located at Oloff Palme street, esq. Núñez de Cáceres, D. N., Dominican Republic.

2.1.2 Mango seeds: The mango seeds (*Mangifera indica* L.) used for this study were supplied by the mango Cluster in the San Cristóbal province. The varieties were Banilejo and Mingolo.

2.1.3 Enzymes: Viscozyme L, Neutrase 1.5 MG and Alcalsae 2.5L. These were supplied by Trisan Dominican, Novozyme's representative in the Dominican Republic. The enzymes were chosen due to their high efficiency in further studies and their wide use in the food industry. (Mehanni, 2021). The trade names of the enzymes and their chemical compositions are listed in Table 1.

Table 1. Chemical and commercial names of the enzymes and their ideal parameters.

Enzymes		Ideal Parameters		Mixture
Comercial name	Composition	T(°C)	pH	Composition (%)
Viscozyme L	Protease	55	6 - 8.5	25
Alcalase 2.5L	Pectinase /Xylanase /Bglucanase /Celulase	50	4.5 - 7	65
Neutrase 1.5 MG	Protease	50	5 - 7	10

(Mehanni, 2021) de (Novozyme, Dinamarca)

2.1.4 Solvents: N-Hexane, 99.9% purity, Fisher Chemical, HPLC Grade, Absolute Ethyl Alcohol, 99.5%, J.T Baker, Analytical Grade. All reagents were supplied by (Industrial Technical Chemist SRL).

2.2 Methods
2.2.1 Obtaining flour from mango seeds
2.2.2 Pretreatment:
The mango seed stones are placed in a 1% w/w citric acid solution to prevent oxidation during the drying process.

2.2.3 Drying:
The mango seed pits are put into the dehydrator. A dehydrator that uses propane gas as fuel was used. TSUNGHSING brand, Model A-200, where the mango seeds were placed at a temperature of 55ºC ± 3ºC, for 24 hours

2.2.4 Grinding:
The dried mango seed stones were ground using a PRO-02 laboratory mill to obtain mango stone meal.

2.2.5 Storage:
Mango bone meal was packed in sealed plastic bags and stored at room temperature until use.

2.2.6 Extraction of oil from the mango seed pit
2.2.6.1 Extraction with Hexane:
Hexane-based solvent extraction is the most widely used methodology to obtain vegetable oil. For the release of the oil contained in the solid matrix in a fluid medium, the mango seeds are placed in compacted bags that come into contact with the pure solvent.

For the soxhlet extraction, 510 mL of hexane was placed in a flat-bottomed flask connected to a soxhlet separator with 25.5 g of mango stone meal. The extraction was maintained for 4 hours, then the hexane was distilled under vacuum using a rotary evaporator (Yamato, RE500) at 50 °C, the extract was kept refrigerated for future studies.

2.2.6.2 Enzymatic extraction:

Enzymatic extraction is an efficient and clean procedure for extracting oil and protein from a wide variety of oilseeds. The enzymes used for each oilseed are chosen based on their different structures.

For the enzymatic extraction, 100 g of mango bone flour were taken and mixed with 1000 mL of water in a 1.5 L beaker for a 1:10 p/p ratio. This mixture was slowly brought to a boil for 3 min and then cooled to room temperature, before being incubated according to the methodology described by (Article).

According to (Puangsri, Abdulkarim, & Ghazali, 2005) cited by (Mehanni, 2021), the pH and temperature were changed at 12 hours with the ideal parameters for each enzyme from table 1.

After 24 hours of incubation, the mixture was centrifuged for 30 min at 4500 RPM using a Beckman Coulter Allegra X-22R centrifuge, and then separated by decantation.

Equation 1: Oil extraction yield

$$Yield\ (\%) = \frac{g\ of\ oil\ by\ \ enzymatic\ extration}{g\ of\ oil\ extracted\ by\ hexane}\ x\ 100$$

Equation 2: % Extraction

$$\%\ Extration = \frac{g\ \ of\ oil}{g\ of\ mango\ seed\ pit}\ x\ 100$$

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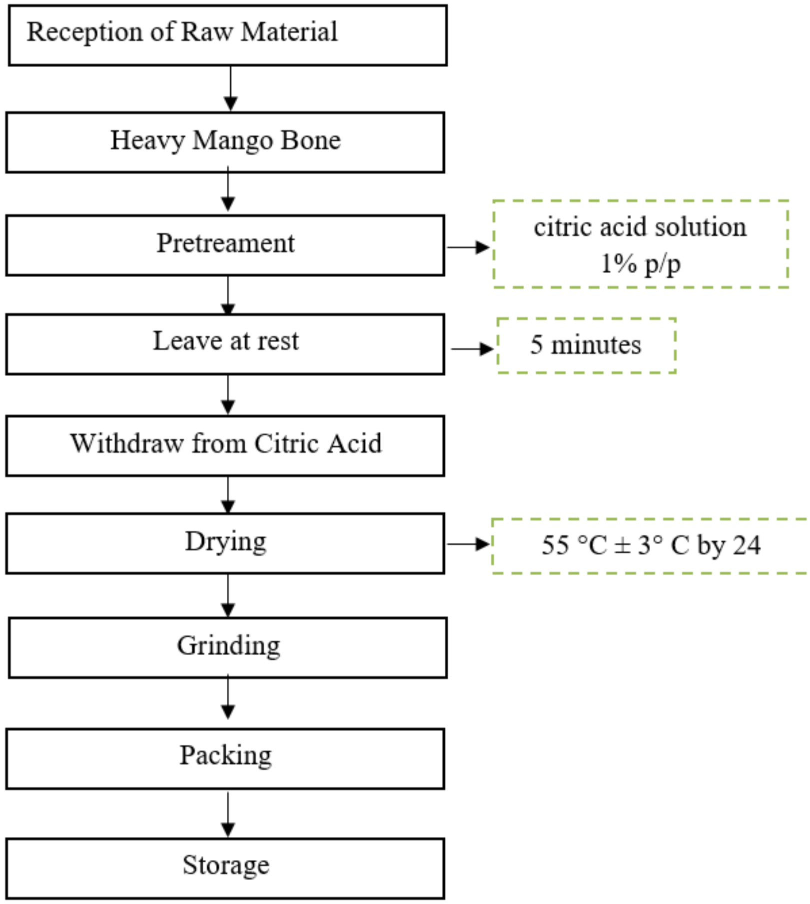

Fig. 1. Flowchart for extraction of mango bone meal

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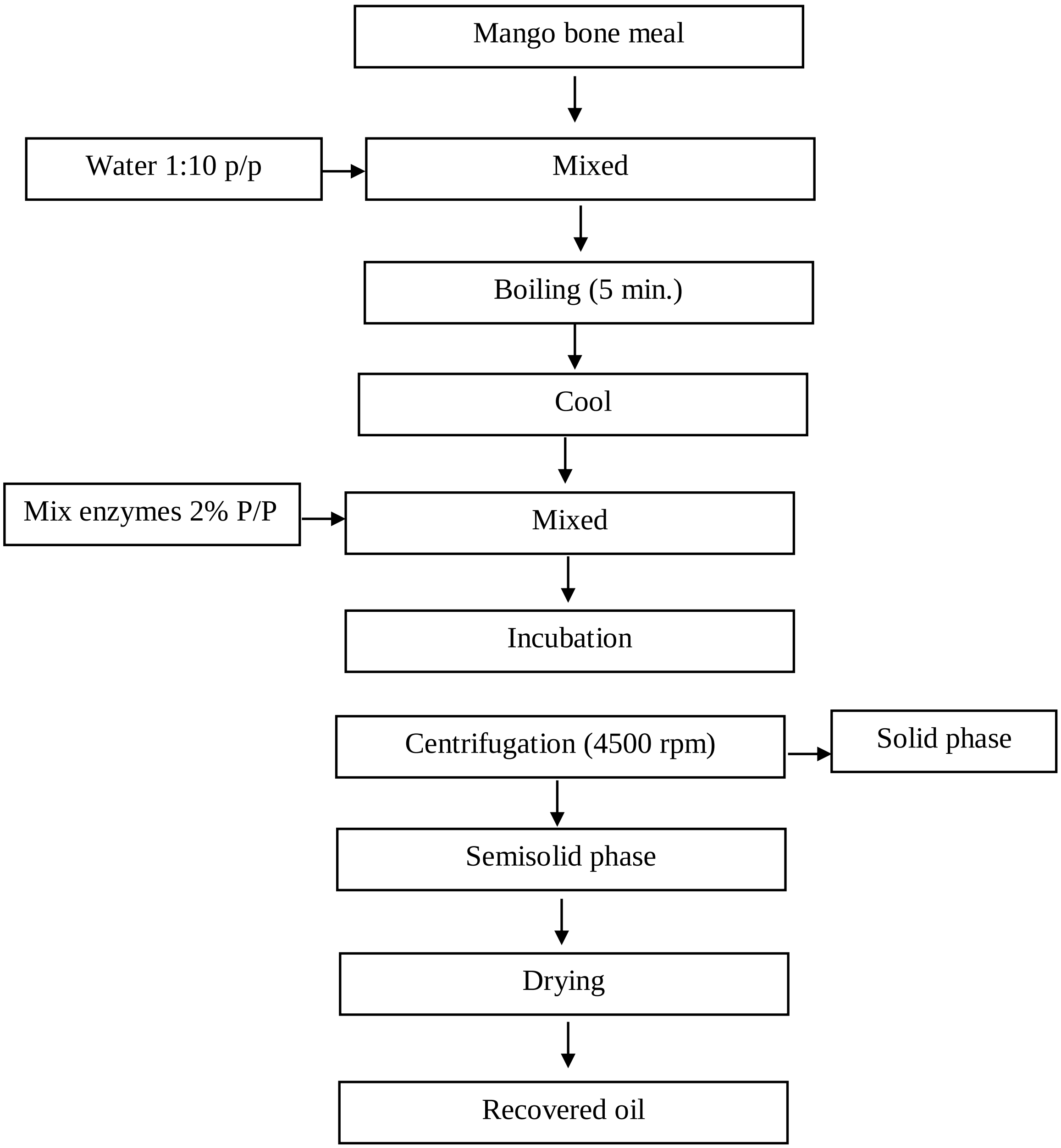

Fig. 2. Flow chart for enzymatic extraction of mango seed oil

3. RESULTS AND DISCUSSION

3.1 Oil Extraction Yield

Table 2. Extraction results and yield

Method	% Extraction	Yield (%)
Hexane	8.366 ± 0.816	-
Enzymatic	0.896 ± 0.037	10.71

Three measurements were made and the mean ± the standard deviation is presented.

The results obtained show that the combination of these enzymes presents a 10.71% yield compared to the enzymatic method (Table 2). These results disagree with those obtained by (Mehanni, 2021), who used the same enzymes and methodology, unlike they mention (modified seed flour), without mentioning what type of modification it refers to.

By centrifuging the enzymatic hydrolysis at 4500 rpm, the mango pit oil (*Mangifera indica* L.) was trapped in a semi-viscous phase with traces of solids. This had to be carried out to dehydration to later separate the oil from the solid phase. This may indicate that it is necessary to centrifuge at more than 4500 rpm to guarantee a complete separation of the solid, aqueous and oil phases.

When boiling the mixture of mango stone flour with water, it presents a gelatinous consistency produced by the partial hydration of the starches present in the flour, it also seems to soften the fibers and allow enzymes to access the cells of the material more easily.

4. CONCLUSION

The percentage of oil extraction using enzymes was low compared to the extraction by hexane. The mango seed was hydrolyzed using a ternary combination of 65% Alcalase, 25% Viscozyme L and 10% Neutrase.

The enzymatic methodology presents an alternative for the extraction of mango seed oil with cosmetic and food applications.

5. ACKNOWLEDGMENT

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