Characterization and Use of *Mangifera indica* L. Seeds from Four Varieties

Paula X. Villanueva, * Yalile C. Ávila, Lina R. Dávila, John J. Méndez, and Walter Murillo Arango

Chemical compositions (fatty acids, total phenolic compounds, flavonoids) and the *in-vitro* biological activities (antioxidant and antimicrobial activity; and growth induction of edible fungal strains) were compared for four varieties of mango seeds. Hexanic extract (HE) showed a fatty acid profile with a higher proportion of oleic acid. In the ethanolic extract were found variable total phenolic contents (103 to 125 mg GAE/g dry weight) and flavonoid contents (0.72 to 0.8 mg QE/g dry weight). This study reports for the first time the presence of procyanidin B1 in ethanolic extracts. The antioxidant activity showed IC_{50} values ranging from 3.09 to 3.42 µg/mL for ABTS•+ and 12.17 to 13.93 µg/mL for DPPH•. The ethanolic extract from the seed of the Yulima variety showed the highest percentages of inhibition against *Staphylococcus aureus.* Residues removed from ethanolic extraction of the seed kernel (EKR) induced the growth of edible fungal strains: *Lentinus crinitus* and *Pleurotus tubarius.* The data obtained show the potential of the seeds from these mango varieties, which could lead to alternative uses in various industry sectors and the use of this agricultural byproduct.

Keywords: Mango seed kernel; Polyphenols; Antioxidant; Flavonoids; Antimicrobial; Fatty acids; Edible fungi

Contact information: Grupo de Investigación en Productos Naturales de la Universidad del Tolima, GIPRONUT, Chemistry Department, Science Faculty, University of Tolima, 730006, Ibagué, Colombia;
** Corresponding author: paulaxvb@ut.edu.co*

INTRODUCTION

Mango is widely produced in the tropical and subtropical regions of the world. It is classified in the fifth position among the main fruits, and it is cultivated in more than 100 countries of the world (Nadeem *et al.* 2016). Mango is one of the most widely consumed and commercialized fruits in the world (Oliveira *et al.* 2016), with a production of approximately 33 million tons (t). In 2017, Colombia produced 280.605 tons of mango (Agronet 2020). In Colombia there are 16 varieties of mango that can be grouped in creole and improved varieties. Común, Manzano, and Mariquiteño varieties are creole. Yulima is an improved variety. These varieties are the most cultivated in the Department of Tolima. This region is positioned as the largest producer in the country, contributing 28.95%, which makes this region an important production area for this crop in the country. Mango fruits are processed for products such as puree, nectar, leather, canned slice and chutney, juices, ice cream, fruit bard, and pies (Yatnatti *et al.* 2014).

During the mango production there is a significant percentage of loss linked to three different periods in the distribution chain: agricultural and postharvest production, storage, and industrial processing (Departamento Nacional de Planeación 2016). A minimum of 15% loss in terms of quality has been estimated, but in periods of high harvest and low

prices, producers experience losses of up to 40% (Fedemango 2017). It has been estimated that global mango production for 2017 reached 47.1 million tons (FAO 2017), and its processing generates between 25 and 40% in losses (Banerjee 2016). During industrial processing most of the biological waste generated from the conversion of raw mango into processed products is associated with the seeds (Dorta *et al.* 2012). The mango seed kernel represents 45 to 75% of this waste, and more than a million tons of seeds are wasted (Leanpolchareanchai *et al.* 2014).

The elimination of mango seed is increasingly difficult, and their disposal is linked to environmental problems (Dorta *et al.* 2012). Mango seed could have valuable compounds that can be exploited by applying new waste recovery strategies (Fernandez-Ponce 2015). It has been proposed that biorefineries could be used as a viable alternative to generate value-added products from biomass used as a raw material, which would prevent loss or the underutilization of the waste (Arora *et al.* 2018). Furthermore, considering that this alternative poses a continuous recovery cycle, it could be proposed as the next step in the mango production system.

Several studies have shown that it is possible to recover compounds with antioxidant, antimicrobial, and antifungal properties from mango seed (Rajan *et al.* 2012; Subbiya *et al.* 2013; Maisuthisakul and Gordon 2014; Dorta *et al.* 2015; Hoyos-Arbeláez *et al.* 2018; Ballesteros-Vivas *et al.* 2019; Melo *et al.* 2019), which generates high hopes for the continued search for new drugs and bioactive components in the food, cosmetic, and pharmaceutical industry (Khammuang, S and Sarnthima, R. 201; Bolla.,2011).

The use and characterization of the seed varieties Común, Mariquiteño, Yulima, and Manzano varieties has never been evaluated in the region. Therefore, this study was carried out to characterize the chemical composition (fatty acids, phenolic compounds, flavonoids) and the *in-vitro* biological activities (antioxidant, antimicrobial, and growth induction of edible fungal strains) of mango seed of this varieties. This study proposes a use of this undervalued waste through biological alternatives.

EXPERIMENTAL

Materials

Four varieties of mango (*Mangifera indica* L.*)* were collected in the municipality of Espinal in the department of Tolima (Colombia): Yulima, Manzano, Mariquiteño, and Común. Pathogen-free fruits were selected during ripening stages 3 and 4 according to Colombian Institute of Technical Standards and Certification (ICONTEC, for its acronym in Spanish) standards 5210 (2003) and 5139 (2002).

Collection and treatment of the material

Seeds from ripe fruits were collected after separating from the pulp. The seeds were washed, cut longitudinally to separately obtain the endocarp and kernel. Endocarp and kernel were dried (40 °C for 24 h) and ground to standardize the particle size in an electric mill into separate flours.

Obtaining extracts

The kernel flours (50 g) of the four varieties were extracted with 200 mL of n-hexane in Soxhlet apparatus for 8 h obtaining hexanic extract (HE). The ethanolic extract (EE) was prepared from the extraction of kernel residues using ethanol in Soxhlet apparatus

for 8 h. The extracts obtained (HE and EE) were evaporated and concentrated using a rotary vacuum evaporator to 45 °C (Büchi, Flawil, Sweden). The extracts were stored together with the extraction kernel waste (EKR) in a freezer at minus 85 °C (Kaltis 390 freezer) until use. The latter was used as a growth inducer for the edible fungal strains.

Fatty acid composition

A weight of 0.170 g of HE was taken for the isolated fatty acid mixture, and 4.0 mL of NaOH in methanol was added. The mixture was refluxed in a water bath at 90 °C for 7.0 min; after this time 5.0 mL of boron trifluoride (BF_3) to 12% in methanol was added, and it was allowed to reflux for another two (2) min. Then 4.0 mL of HPLC grade heptane was added, and it was allowed to reflux for another (1) min. The solution was cooled, and 100.0 mL of saturated NaCl solution was added. The upper (organic) phase and sodium sulfate anhydrous were separated. The samples were subsequently injected into an Agilent® 6890 brand gas chromatograph equipped with a flame ionization detector (FID), under the following chromatographic conditions: injector and a 50.0 m capillary column DB-23, internal diameter 0.25 mm, film thickness 1.4 µm. A total of 1.0 µL was injected in all cases; the injector temperature was 220 ° C, the temperature of the detector was 240 ° C, the flow rate of the carrier gas (H2) was 40.0 cm /s, with a column pressure of 23.04 psi. The gas mixture in the detector was: Hydrogen flow: 45 mL / min, air flow: 450 mL / min, was used as gas makeup (N_2) with a flow of 45 mL / min, a 50:1 split ratio was used in a ramp run temperature: Initial column temperature: 200 ° C (isothermal for 10 min) the temperature rises to 220 ° C with a rate of 2.0 ° C / min (isothermal for 4.0 min).

Antioxidant activity of the ethanolic extracts

The antioxidant activity was tested by adopting an ABTS and DPPH assay (Braca *et al*. 2002; Marquina *et al*. 2008). The DPPH and ABTS scavenging activity were analyzed using a UV-VIS 96-well microplate reader (Multiskan® GO, Thermo Scientific, Vantaa, Finland). The results were expressed as inhibitory concentration value (IC_{50}), which is the concentration of the sample stabilizing the $DPPH^{\bullet}$ or $ABTS^{\bullet+}$ radicals by 50%. Calibration curve was obtained using different concentrations (0.0312 to 1 µg/mL for DPPH and 0.0039 to 0.0625 µg/mL for ABTS) of Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) as standard. All these methods were conducted in triplicate.

Antimicrobial activity of the ethanolic extracts

The microdilution method was performed and the following microorganisms were used for these experiments: *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC29213), *Rhizopus sp.*, and *Fusarium sp*. The antibacterial and antifungal activity assays were performed using the M7-A8 and M38-A2 methodologies, respectively, according to the Clinical and Laboratory Standards Institute (CLSI). The extracts were tested at the following concentrations: 2000, 1000, 500, 250, 125, 62.5, and 32 µg/mL. Positive controls of 250 µg/mL oxytetracycline and 500 µg/mL ketoconazole were used. For the antibacterial activity, bacterial strains were cultured overnight at 37 °C on Brain Heart Infusion Nutritious broth and adjusted to a final concentration of 1 × 108 colony forming units (CFU)/mL (0.5 nephelometric turbidity units - McFarland scale), and the optical density was measured after 0, 6, 18, and 24 h of incubation at a wavelength of 460 nm. For the antifungal activity, pathogen (*Rhizopus* sp. and *Fusarium* sp.) spore suspensions were prepared at a concentration of $1x10^4$ conidia/mL and the optical density was measured after 0, 18, 24, 36, 48, and 72 h of incubation at wavelength of 595 nm. The

absorbance values (optical density-OD) were measured spectrophotometrically using a Multiskan® GO UV/VIS microplate reader (Thermo Scientific). Inhibition percentages (%INH) were calculated using Eq. 1,

$$\% \text{ INH } = \frac{\text{ODC} - \text{ODT}}{\text{ODC}} \text{ X100} \quad (1)$$

where ODC is the optical density of the control, ODT is the optical density of the treatment, and %INH is the percentage of inhibition.

Chromatographic analysis of the phenolic composition

The phenolic composition of the kernel EE from the four varieties was determined by UPLC chromatography. The UPLC chromatographic analysis was performed in the ACQUITY UPLCTM instrument (Waters, Milford, MA, USA) coupled with an ACQUITY UPLCTM PDA diode array detector and an ACQUITY UPLCTM FLR fluorescence detector according to the methodology described by Delpino-rius *et al.* (2015). The standard curve was prepared from a mixture of procyanidin B1, procyanidin B2, epicatechin, hyperoside, rutin, isoquercitrin, and quercitrin (0.05 μg/mL, 0.1 μg/mL, 1 μg/mL, 5 μg/mL, and 10 μg/mL, respectively).

Quantification of phenolic compounds and flavonoids

The kernel flours (500 mg) of the four varieties were extracted with 7.5 mL of acetone:water (6:4) solution in maceration for 2 h. Acetone was allowed to evaporate for half an hour and centrifuged at 11.000 rpm for 15 min. After centrifugation, an aliquot of the supernatant was mixed with methanol (5 mL) to obtain the extracts. The quantification of the phenolic compounds and flavonoids was determined according to the methodology described by Rodriguez *et al.* (2012) with some modifications. Solutions of gallic acid (Sigma-Aldrich®) (25 to 800 μg/mL) and quercetin (2.5 to 30 μg/mL) were used to construct the calibration curves. The results were expressed as milligram equivalents of gallic acid/gram of dry material (mg GAE/g) and milligrams of quercetin/g of dry material (mg QE/g).

Growth of edible fungi in mango seed waste

The mycelia growth of *Lentinus crinitus* (L.) Fr. and *Pleurotus tubarius* (Pat.) Peglers were evaluated in different treatments with mango endocarp (ME) and extraction kernel residues (KR). Five culture media agars (ME Yulima-T1, ME Manzano-T2, ME Mariquiteño-T3, ME Común-T4 and extraction kernel residues-T5) were use. Fungi mycelial growth was tested in Petri dishes following the methodology proposed by Martínez *et al.* (2015). The ME and KR were used for determination of proximate analysis and mineral element composition according to AOAC (1995). Person correlation analysis was performed between the growth rate and the variables of the proximal and mineral composition.

Statistical analysis

ANOVA was performed for each calculation, and the means with their respective standard deviations (SD) are presented in tables. When there were significant differences between the means of each treatment ($P < 0.05$), the Fisher's least significant difference (LSD) a posteriori test was performed. To analyze the mycelial growth of edible fungi due to each treatment, the rate of growth of each treatment was evaluated and the significant

differences between the treatments were checked. These analyzes were performed using the R-study program.

RESULTS AND DISCUSSION

Table 1 presents the fatty acid profiles of the four mango seed varieties. Oleic and stearic acid are shown in higher proportion. Yulima, Manzano, and Mariquiteño varieties showed values of oleic acid of 44.73, 46.1, and 44.78%, respectively, followed by stearic acid with values of 40.4, 39.74, and 38.0%, respectively. The Común variety presented a different proportion with a higher stearic acid content of 44.70% and a lower percentage of oleic acid, at 41.51%. Palmitic, linoleic, and linolenic acids were found in lower percentages in all the mango varieties. These results can be compared with the reports by Mariod *et al.* (2017).

Table 1. Relative Composition of Fatty Acids Derived from the *Mangifera indica* Seed Kernel

Fatty Acids (%)	Yulima	Manzano	Mariquiteño	Común
Lauric	0.00646 ± 0.00009	0.0033 ± 0.0005	0.00552 ± 0.00005	0.00453 ± 0.00001
Myristic	0.025 ± 0.001	0.023 ± 0.003	0.034 ± 0.006	0.022 ± 0.002
Palmitic	6.385 ± 0.005	6.643 ± 0.003	7.7 ± 0.2	5.720 ± 0.004
Stearic	40.395 ± 0.009	39.7338 ± 0.0006	38.0 ± 0.2	44.70 ± 0.07
Oleic	44.7214 ± 0.0006	46.11 ± 0.02	44.78 ± 0.07	41.51 ± 0.06
Linoleic	3.682 ± 0.003	3.4518 ± 0.0002	5.70 ± 0.07	3.92 ± 0.01
Linolenic acid	0.4184 ± 0.0006	0.2385 ± 0.0008	0.306 ± 0.006	0.304 ± 0.004
EPA[a]	0.501 ± 0.001	0.402 ± 0.002	0.3782 ± 0.0009	0.447 ± 0.002
DHA[b]	0.00916 ± 0.00006	0.0078 ± 0.0002	-	-

±: Standard deviation; EPA[a]: eicosapentaenoic acid; DHA[b]: docosahexaenoic acid. Each value represents the mean ± SD (n = 3).

The most used active ingredients in cosmetics are oils from seeds that are rich in unsaturated fatty acids (Alcalde 2007), indicating that fatty acids in mango can be used for different applications, including food. For example, Kittiphoom (2012) and Jahurul *et al.* (2014) report that mixtures (80:20) of mango lard and palm stearin could be used to produce butter resistant to hot climates and be used as a substitute for cocoa butter. Beyond the food industry, in the cosmetics industry, there is great demand for ingredients in soaps, moisturizing creams, lubricants, hair softeners and formulations for lipstick and phytomilk (Méndez Arteaga *et al.* 2013).

Phenol, Flavonoid, and Procyanidin B1 Contents

The total phenolics contents detected in the four mango variety seed varieties are shown in Table 2. The highest values were detected in Yulima and for the other varieties the contents were statistically similar with values from 103 to 110 mg GAE/g. The four varieties evaluated had higher phenolic contents than those found in other studies with

cultured mangoes of different origins (Adilah *et al.* 2018; Melo 2019; Castro-Vargas *et al*. 2019). However, compared with other reports, the present values are considered much lower because they have up to two-, three-, and fourfold more phenolic contents than previously studied varieties (Khammuang and Sarnthima 2011; Sogi *et al.* 2013; Dorta *et al.* 2014; Patiño-Rodriguez 2019). These variations can be attributed to factors such as genetic characteristics, environment, maturity stage, and agricultural practices (Torres-León *et al.* 2016).

Table 2. Content of Phenolics, Flavonoids, Procyanidin B1 Compounds and Antioxidant Activity of Ethanolic Extracts

Mango variety	Total phenolics content[a]	Total flavonoid content	Procyanidin B1 (µg/g)	Antioxidant activity (*IC50 µg/mL)*	
				ABTS•+	DPPH•
Yulima	125 ± 2.1 a	0.8 ± 0.04	0.407 ± 0.0058 c	3.4	13.9
Manzano	110 ± 4.6 b	0.8 ± 0.02	0.152 ± 0.0402 b	3.4	13.0
Mariquiteño	103 ± 8.3 b	0.77 ± 0.2	0.065 ± 0.0024 a	3.4	12.2
Común	107 ± 8.2 b	0.72 ± 0.07	0.146 ± 0.01900 b	3.1	12.2

[a]mg GAE/g dry weight (GAE: gallic acid equivalent); [b]mg QE/g dry weight (QE: quercetin equivalent). Means with different letters represent significant differences between varieties ($p < 0.05$) with Fisher's least significant difference (LSD) test for each column.

The flavonoid contents of each of the varieties was similar, with values ranging from 0.72 to 0.8 mg QE/g dry weight. In contrast, Dorta *et al.* (2014) compared different mango varieties with respect to their flavonoid contents and found minimal differences for the Gomera, Keitt, and Sensation varieties which had flavonoid contents of 0.72, 0.95, and 1.17 mg CE/100 g dry weight, respectively.

Despite the high phenol contents found in the tested samples, the results suggest that flavonoids constitute a minimal proportion of these compounds. However, flavonoids are associated with a broad spectrum of health-promoting effects and are indispensable components in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic properties, together with their ability to modulate cellular enzyme function (Panche *et al.* 2016).

The chromatographic profiles of the *Mangifera indica* seed ethanolic extracts were very similar in composition. Thus, it was possible to identify signals at the retention times (RTs) ranging from 7.99 min to 16.10 min. This was in contrast to the patterns observed in the UV and fluorescence spectra. These data made it possible to characterize flavan-3-oles as the main family of phenols.

Within this family, procyanidin B1 is reported for the first time in the mango kernel of the studied varieties, which is contrast to previous reports showing that it was limited to mango pulp (Rue *et al.* 2017). Procyanidins are condensed flavan-3-ols that constitute an important group of polyphenols due to their bioactivity. Procyanidin B1 shows chemopreventive potential against cancer (Lee 2017), antioxidant activity (Muselík *et al.* 2007) and anti-inflammatory (Xing *et al.* 2015), antidiabetic (Gonzalez-Abuin *et al.* 2015)

and antiviral (Li *et al.* 2010) properties. Given its presence in the kernel of the four varieties studied, the potential of this byproduct stands out in terms of the integrative use of the fruit.

Bioactivity of Ethanolic Extracts from Mango Seed

The ethanolic extracts derived from the seeds of the four varieties showed antiradical activity against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ at low concentrations (Table 2). The IC_{50} ranged from 3.1 to 3.4 μg/mL against $ABTS^{\bullet+}$ and between 12.2 and 13.9 μg/mL against $DPPH^{\bullet}$, reflecting the similarity between the varieties. There are reports supporting that there exists a relation between the phenol contents and antioxidant potential of extracts of plants (Hossain *et al.* 2014; Das *et al.* 2015; Khanal *et al.* 2015), which would explain the remarkable antioxidant capacity of the four varieties given their high phenol contents. The mango seed ethanolic extract is among the top four of 100 extracts evaluated with regard to its antioxidant capacity (Torres-León *et al.* 2016).

Additionally, the results from the first method (IC_{50}: 3.1 to 3.4 μg/mL) are consistent with the results of the method proposed by Nakpanich *et al.* (2017) for the *Mangifera indica* seed methanolic extract, which presented an IC_{50} of 3.4 μg/mL. Similarly, Khammuang and Sarnthima (2011) report ethanolic extract values of 4.13 and 4.8 μg/mL for the Chok-a-nan and Nam-dok-mai varieties, respectively, which come from a common area.

The antimicrobial potential of the extracts was also evaluated**.** The supplementary information shows the inhibition percentages generated by the ethanolic extracts of the four varieties against the microorganisms tested. In the case of the Yulima variety, inhibition percentages greater than 50% were generated against *S. aureus* at the lowest concentration evaluated and against *E. coli* and *Rhizopus* sp. at concentrations greater than 250 μg/mL. In contrast, the Manzano, Común and Mariquiteño varieties showed inhibition percentages greater than 50% against *S. aureus* and *Rhizopus* sp.

The results obtained can be compared with those other studies in which extracts with different mango kernel polarities and their antimicrobial activity have been used. For example, Abdalla *et al.* (2007) showed that the mango kernel methanolic extract inhibited *E. coli* growth at a concentration of 400 μg/mL. The mango kernel methanolic extract has been reported to inhibit a wide range of microorganisms at a concentration of 200 μg/mL (Engels *et al.* 2011), which is consistent with the results obtained in this study, specifically for *S. aureus*, at concentrations between 125 and 250 μg/mL. As stated by Vaghasiya *et al.* (2011), the presence of elevated levels of tannins and phenolic compounds in the extract could promote antimicrobial bioactivities. The phenol contents may play an important role in antimicrobial activity; however, it is necessary to predict with certainty which group of metabolites is generating this activity.

Some authors, such as Shabani and Sayadi (2014), have reported a concentration of 25 μg/mL to inhibit important bacteria such as *Salmonella enteritidis*, *Klebsiella aerogenes*, and *E. coli*, among others. Kabuki (2000) reported inhibition of *E. coli* at 2500 μg/mL. By contrasting these results with those obtained in this study, it can be predicted that the ethanolic extracts obtained from the mango kernel have an interesting potential to inhibit this type of microorganism at concentrations lower than those previously reported.

Related to the percentages of inhibition against *Rhizopus sp.* (supplementary information), it was found that all of the extracts inhibited *Rhizopus sp.* between 87 and 89.6% at a concentration of 1000 μg/mL, with a dose-response effect. Few studies have evaluated mango seed for this biological activity; among them, Dorta *et al.* (2016) reported minimum inhibitory concentrations (MICs) for the three mango cultivars as a function of

the total phenol concentration in the extract. These authors determined MICs from 0.1 to 5 mg GAE/mL against 17 fungi, demonstrating that there is a relationship between the phenolic composition (high levels of proanthocyanidins, gallates and gallotannins), antifungal activity and the ability to inhibit lipid peroxidation. Among the few reports on the antifungal activity of mango kernel extracts, none emphasize the organisms used in the present study; therefore, the results found here are the first to report antifungal activity by the ethanolic extracts obtained from the seeds of mango varieties.

Extraction Kernel Waste as a Substrate for the Growth of Edible Fungi

Mango waste from the varieties under study has been explored in their various forms in the recovery of important metabolites. However, this study examined the viability of seed flour (press cake) and the endocarp as a substrate for the mycelial growth of edible fungi. This alternative was proposed given that mushrooms can colonize a wide variety of lignocellulosic substrates (Martínez *et al.* 2015b; Coello-Loor *et al.* 2017; Dávila *et al.* 2020). Table 3 shows the growth rate of two strains on extraction kernel waste of mango. It was observed that all wastes induced the growth of edible strains, and in some cases the growth rate was higher than that of the potato dextrose agar (PDA) basal medium.

Table 3. Mycelial Growth of Edible Fungi on Mango Waste

Treatment	Growth Rate (GR) (cm/day)	
	Lentinus crinitus	*Pleurotus tubarius*
T1(Yulima)	1.38 ± 0.072 a	1.71 ± 0.15 b
T2 (Manzano)	1.39 ± 0.036 a	1.52 ± 0.074 c
T3 (Mariquiteño)	1.19 ± 0.086 b	1.38 ± 0.072 d
T4 (Común)	1.41 ± 0.058 a	1. 59 ± 0.056 bc
T5 (PC)	0.32 ± 0.014 c	1. 68 ± 0.13 bc
(Control) PDA	1.17 ± 0.029 b	1. 71 ± 0.09 ab
Each value is expressed as the mean ± SD (n = 3) (ANOVA $p < 0.05$). T5 extraction kernel waste: Mango seed flour remaining after extraction. Means with different letters represent significant differences between varieties ($p < 0.05$) with Fisher's least significant difference (LSD) test.		

Mycelial growth varied depending on the treatment used, with the waste of the Común variety inducing the fastest growth of *L. crinitus* and the Yulima waste inducing the greatest growth by *P. tubarius.*

These differences may be due to the composition of the waste, as observed in Fig. 1, which shows the correlation between the composition of the medium and the growth rate. This figure indicates the Pearson product correlations between each pair of variables. The range of these correlation coefficients is from -1 to +1, which measures the strength of the linear relationship between the variables represented in red to yellow. Additionally, the figures show, in circles, only those variables that presented a correlation with p values less than 0.05.

In this sense, the growth rate of *P. tubarius* is negatively related to the protein, phosphorus, ash, and nitrogen contents and positively related to the fiber, calcium and sodium contents. For *L. crinitus*, mycelial growth is positively related to the iron content. However, it should be clarified that there are other factors that may influence the growth of edible fungus strains.

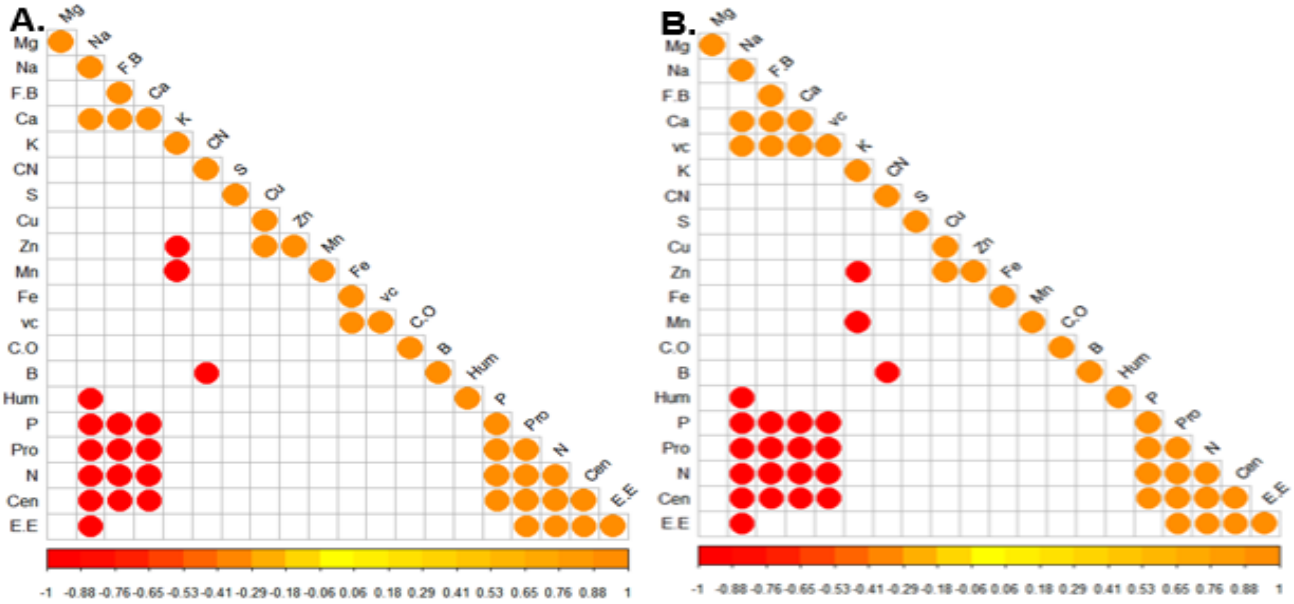

Fig. 1. A and B. Correlation matrix of the Pearson between wastes remaining composition variables and growth rate of *L. crinitus* and *P. tubarius,* respectively. Light orange color indicates positive relation of the variables, red-inverse correlation. Only significant correlations are filled with color and circles (confidence = 0.95).
Hum: Humidity (%), Ash: ash%), Pro: Protein (%), EE: Ethereal extract (%), FB: Crude fiber (%), CO: Organic carbon (%), N: Nitrogen (%), Ca: Calcium (%), Na: Sodium (mg/kg), K: potassium (%), Mg: Magnesium, Cu: Copper (mg/kg), Zn: Zinc (mg/kg), Fe: Iron (mg/kg), Mn: Manganese (mg/kg), B: boron (mg/kg), P: phosphorus (%) and S: Sulfur (%).

This type of test is important to select the best waste or combination of wastes to process these wastes into other value-added products, such as the production of basidioma for human consumption (Motato *et al.* 2006; Martínez *et al.* 2015; Oviedo *et al.* 2016) or other products of general interest, including waste pressed into cakes with the fungus as soil improvers or as animal feed (Hanafi Fatimah *et al.* 2018).

The use described in this research is performed in an integrated manner, as summarized in Fig. 2, where the system processes the seed product wastes (kernel and endocarp) of the four varieties. The flour serves as a source of metabolites of interest, including fatty acids and phenolic compounds, the latter of which has important bioactivity, such as antioxidant and antimicrobial activities. Moreover, the fatty acids present in the wastes have a similar composition to cocoa seed fats, which are used in the food and cosmetic industry. This potentiates the use of mango fatty acids in these fields because they could be even more economical to obtain. Additionally, the waste in the press cake and the remaining endocarps were used as substrates for the growth of edible fungi, which can colonize this type of substrate for the production of basidioma for human consumption or the material biodegraded by the fungus, as compost or animal feed. This system could be used for the creation of a collection center for this type of waste material whose similarity in composition and bioactivity would not involve its separation. Consequently, an added-value chain could be generated that could benefit the producer and reduce environmental problems due to waste disposal. This research provides information for management alternatives and for the final disposal of mango seeds, especially varieties that are found in the region with a variety of bioactivities and compositions that generate value-added products.

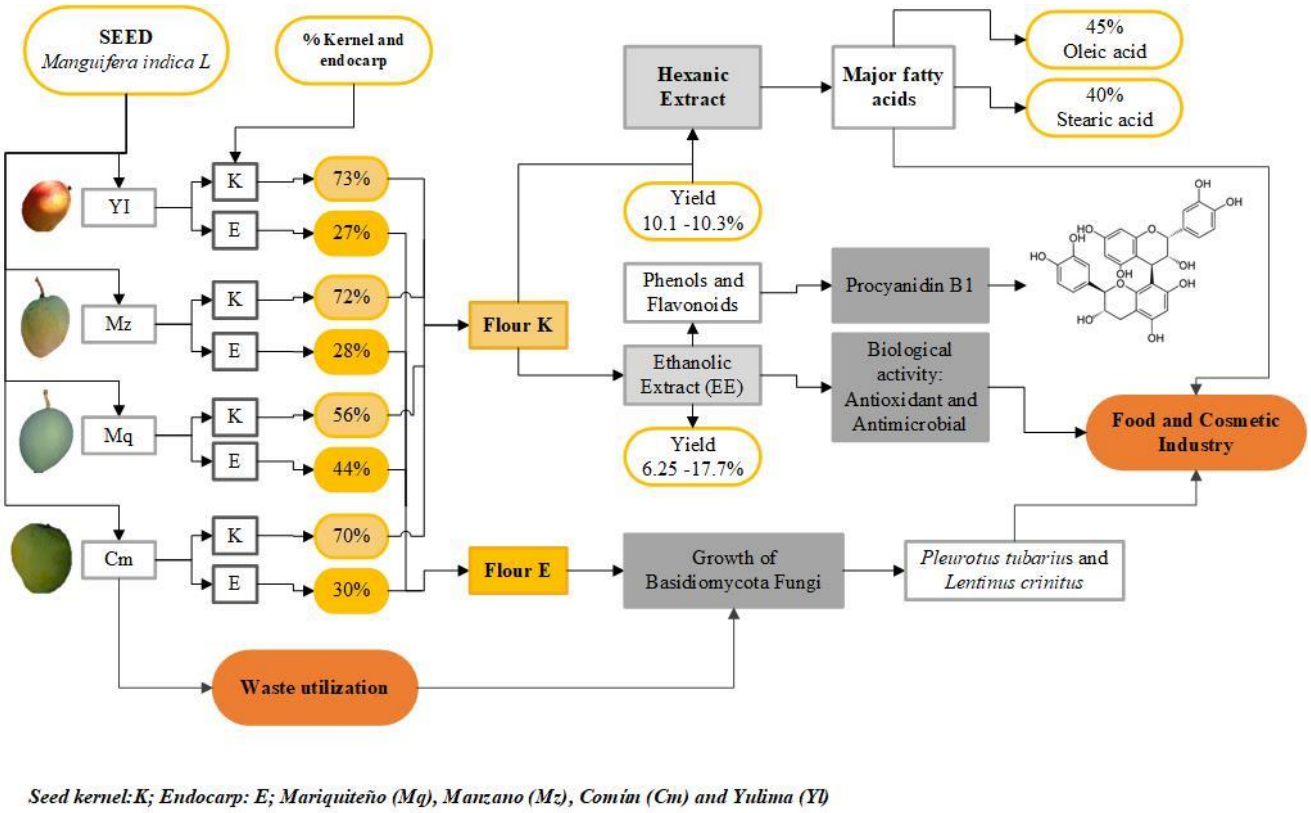

Fig. 2. Flow chart on the characterization and use of mango seeds from four varieties cultivated in the Department of Tolima, Colombia

CONCLUSIONS

This research reports the characterization of the mango seed and its potential as source of bioactive compounds as the high phenolic compounds and unsaturated fatty acid contents. The presence of procyanidin B1 was identified for the first time. The antioxidant and antimicrobial activity of ethanol extracts is highlighted.

The use of mango seed as an inducer of fungal growth provides alternative uses and benefits of the waste generated from these mango varieties, which has not been studied extensively. This opens a door to generate more studies of this kind where the generation of alternative income to mango growers and economic feasibility can be verified.

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SUPPLEMENTARY INFORMATION

Table S1. Percentage Inhibition of Ethanol Extracts in Bacteria and Mushrooms

Mango variety	Concentration (µL/mL)	% INH *E. coli*	% INH *S. aureus*	% INH *Fusarium sp*	% INH *Rhizopus sp*
Yulima	2000	-	-	-	-
	1000	55.92 ± 3.12	62.70 ± 5.80	40.92 ± 6.27	89.29 ± 0.63
	500	55.50 ± 0.70	92.42 ± 1.16	23.20 ± 3.57	76.58 ± 16.16
	250	57.60± 1.19	98. 23 ± 1.23	15.65 ± 8.36	33.91 ± 2.31
	125	-	94.58 ± 2.14	15.86 ± 5.97	25.53 ± 3.95
	62.5	-	82. 50 ± 4.74	18.41 ± 7.67	2.68 ± 4.13
	32	40.85 ± 4.6	62.18 ± 11.56	16.46 ± 6.99	20.94 ± 7.60
Manzano	2000	-	-	-	-
	1000	58.74 ± 4.4	-	45.77 ± 7.94	89.60 ± 2.11
	500	54.63 ± 1.25	86.25 ± 3.52	17.95 ± 7.0	87.56 ± 17.24
	250	52.84 ± 2.80	97. 67 ± 0.71	18.12 ± 3.65	70.55 ± 16.52
	125	-	87. 56 ± 4.37	17.84 ± 2.15	39. 06 ± 5.3
	62.5	-	78.29 ± 8.05	10.72 ± 3.26	37.31 ± 4.75
	32	38.46 ± 5.75	-	22.32 ± 3.29	21.72 ± 5.53
Mariquiteño	2000	-	-	-	-
	1000	35.47 ± 3.67	71.73 ± 9.31	37. 65 ± 5.63	87.84 ± 6.30
	500	54. 63 ± 2.49	86.17 ± 3.01	18.25 ± 3.21	59.63 ± 10.11
	250	34.18 ± 4.16	97.84 ± 1.19	19.67 ± 3.72	27.45 ± 8.22
	125	-	95.69 ± 3.17	17.19 ± 4.83	22. 04 ± 1.68
	62.5	-	83.62 ± 6.88	18.72 ± 4.67	19.03 ± 7.08
	32	17.00 ± 12.41	-	18. 32 ± 3.5	26.68 ± 8.58
Común	2000	-	-	-	-
	1000	33.30 ± 2.15	-	41.31 ± 3.58	87.08 ± 1.30
	500	41.19 ± 6.92	75.56 ± 4.86	28.73 ± 1.35	78.73 ± 19.59
	250	40.02 ± 1.56	93.78 ± 1.71	23.13 ± 4.48	41.02 ± 8.04
	125	-	97.26 ± 1.03	16.62 ± 10.62	25.93 ± 15.44
	62.5	-	91. 71 ± 4.21	20.13 ± 4.10	30.82 ± 7.20
	32	16.70 ± 7.18	55.53 ± 7.21	19. 16 ± 6.48	27.25 ± 11.21