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EVALUADORES

El Comité Editorial dentro de sus políticas, envía los artículos a especialistas, con el fin de que sean revisados. Sus observaciones en adición a las que hacen los editores, contribuyen a la obtención de una publicación de reconocida calidad en el ámbito de las Ciencias Agrarias. Sus nombres son mencionados como una expresión de agradecimiento.

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Influence of plant density on fruit and foliar nutritional composition for Hass avocado in Colombia

Influencia de la densidad de plantío del aguacate Hass sobre el contenido de nutrientes en frutos y hojas en Colombia

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ABSTRACT

This study aimed to determine the effect of plant density on avocado fruit and leaf nutritional composition in commercial orchard *cv.* Hass in the department of Antioquia, Colombia. The experimental design was a randomized complete block with three replicates. The treatments consisted of six plant densities (204, 278, 333, 400, 625, and 816 tree ha^{-1}), and each experimental unit consisted of six 9-year-old trees. The 333 and 400 trees ha^{-1} plant densities presented the highest leaf concentrations of N, P, Ca, Mg, S, Zn, and B and fruits' lowest saturated fatty acid contents. The nutritional balance index for N, Ca, Mg, S, Fe, Mn, Zn, and B was significantly affected by plant densities. The plant density significantly does not affect K, Ca, S, and Fe concentration in fruits, and the percentage of avocado fruits by size showed no significant differences due to plant distances.

Keywords:
Fruit quality
Harvest season
Main harvest
Planting system
Secondary harvest
Tree spacing

RESUMEN

El presente estudio tuvo como objetivo determinar el efecto de la densidad de plantío sobre la composición nutricional de frutos y hojas de aguacate en un huerto comercial del *cv.* Hass en el departamento de Antioquia, Colombia. Se utilizó un diseño experimental bloques completos al azar con tres repeticiones. Los tratamientos consistieron en seis densidades de plantío (204, 278, 333, 400, 625 y 816 árboles ha^{-1}) y la unidad experimental estuvo compuesta por seis árboles de 9 años de edad. Las densidades de plantas de 333 y 400 árboles ha^{-1} presentaron las mayores concentraciones foliares de N, P, Ca, Mg, S, Zn y B y los menores contenidos de ácidos grasos saturados en frutos. El índice de balance nutricional para N, Ca, Mg, S, Fe, Mn, Zn y B se vio afectado significativamente por las densidades de las plantas. La densidad de plantas no afectó significativamente la concentración de K, Ca, S y Fe en los frutos, y el porcentaje de frutos de aguacate por calibre no mostró diferencias significativas debido a las distancias de las plantas.

Palabras clave:
Calidad del fruto
Época de cosecha
Cosecha principal
Sistemas de siembra
Cosecha secundaria
Espaciamiento entre árboles

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World avocado production in 2021 was 8,865,672 tons, and Colombia achieved 11.28% of the total avocado production. Mexico had 26.40%, Peru 8.95%, and the Dominican Republic 7.30%. Regarding the harvested area, Colombia ranked second (10.97%), behind Mexico (26.40%) (FAO 2023). In the last decade, Colombia increased the harvested area by 335%, going from 21,592 ha (2010) to 94,111 ha (2021). In the same period, production went from 205,443 tons to 976,617 tons, representing an increase of 381% (FAO 2023). However, even though the potential yield of the avocado tree is 32.5 t ha^{-1}, the average world yield was 10.12 t ha^{-1}, presenting a 70% gap to the potential yield of the species for the year 2020 (Gazit and Degani 2013; FAO 2023).

Avocado commercial orchards are traditionally established at wide spacing, between 7x7 m (204 trees per ha) and 10x10 m (100 trees per hectare). The yield per unit area is low during the first few years after planting, which increases until the trees begin to shade each other. The yields decrease after 5 to 10 years; nonetheless, there is great interest in using high-density plantations to increase productivity and yields, especially in the early life of orchards (Menzel and Lagadec 2014). In avocados, the challenge is to reduce the time between planting and full canopy development and maintain orchard productivity and fruit quality once the tree reaches total growth. When establishing an orchard, the choice of planting rate determines the time it takes for the canopy to develop fully, achieving maximum light interception (Whiley et al. 2013). Thus, optimal plant density improves the photosynthetic canopy yield by increasing the light penetration to lower leaves in the canopy, which is beneficial for yield and fruit quality (Ding et al. 2022). There are many options for plant density and orchard design for a given environmental, social, and economic condition. The choice must aim at an appropriate balance between simplicity and complexity that corresponds to the ability to manage and maintain the orchard (Whiley et al. 2013).

Recently, different plant densities, especially the high ones, have been used without evaluating them under Colombian conditions, where temperature and relative humidity limit the continuous development of trees. The topography, cultivar, growing systems pruning, and driving have become critical factors in determining the optimal tree spacing and orchard design (Bernal-Estrada and Díaz-Díaz 2020). So, there has been a lot of interest in using high-density plantations to increase yields, fruit quality, and productivity, especially in the early life of orchards (Menzel and Lagadec 2014). Therefore, the improvements in the fruit quality caused by changing cultivation strategies have been little studied in recent years (Salazar-García et al. 2019). The avocado cultivar and the management practices and conditions where it is cultivated influence the fruit's nutrient concentration (Salazar-García et al. 2019). This is important since the leaf concentration and balance of nutrients at the time of harvest of the avocado fruit is fundamental to ensure the yield and quality of avocado (Arpaia et al. 2015; Salazar-García et al. 2019).

Proper plant density not only helps obtain a high yield and increases fruit quality but also saves labor and production materials (Jovicich et al. 2004); determining the appropriate plant density could provide important economic benefits for growers (Ding et al. 2022). Numerous studies have proven that changing plant density impacts yield (Calori et al. 2017; Haque and Sakimin 2022; Cano-Gallego et al. 2023). Colombia has high genetic and agroecological variability in which avocados are grown, which generates both heterogeneous production systems and management agricultural practices heterogeneity influencing their yield and fruit quality (Carvalho et al. 2014; Tamayo et al. 2018). Although planting high densities has the potential to obtain avocado higher yields than traditional plantations, especially in the first years of production (Menzel and Lagadec 2014), very little is known about plant density effects elements in the canopy. For the above, the present study aims to determine the effect of plant density on fruit and leaf nutritional composition in commercial orchards of Hass avocado in the department of Antioquia, Colombia.

MATERIALS AND METHODS

Location

This research was carried out in two 9-year-old commercial orchards of avocado *cv.* Hass in Antioquia, Colombia, for three consecutive years (2019-2021). The avocado trees planted were grafted on creole rootstock with a scion bud of *cv.* Hass and a minimum of five growth points (standard procedure). The first orchard was located in the Rionegro municipality at 06°5'56.8"LN and 075° 26' 21.9" LW (2,200 meters above sea level (masl)). The second one was in El Peñol municipality at 06°11'28.4"LN and 075° 14' 34.4" LW (2,100 masl). The WatchdogTM 2000 portable weather stations (Spectrum Technologies, 3600 Thayer Court, 107 Aurora, IL 60504) recorded climatic variables in each location. Rionegro presented a mean temperature of 17.2 °C, a maximum of 23.8 °C, and a minimum of 13.0 °C, with an annual rainfall of 1,800 mm. In El Peñol, a mean temperature of 18.5 °C, a maximum of 23.0 °C and a minimum of 14.9 °C was recorded, with an accumulated annual rainfall of 1,921 mm. According to Belda et al. (2014), the region's climate is Cw subtropical dry-winter, according to Köppen's classification. The soil of the experimental area is representative of the region, being classified as an Andosol according to FAO World Reference Base classification (Delmelle et al. 2015).

Experimental design

A randomized complete block experimental design with three replications was used. The experiment unit consisted of 15 *cv*. Hass avocado trees and the treatments of six plant densities. The plant densities (trees ha^{-1}) evaluated were: 204 (7x7 m), 278 (6x6 m), 333 (6x5 m), 400 (5x5 m), 625 (4x4 m) and 816 (3.5x3.5 m).

Five harvest seasons were carried out between 2019 and 2021. Three main harvests (2019M, 2020M, and 2021M) and two secondary harvests (2020S and 2021S). The main harvest (February-March flowering period) was carried out between December and January (next year), and the secondary (mitaca) harvest (August-September flowering period) was carried out in June-July (next year).

Soil analysis

The soil nutrient availability was carried out after each harvest (2019M, 2020S, 2020M, 2021S, 2021M). Subsamples below the tree canopy and at a depth of 0-30 cm were taken for chemical analysis. The results of the soil analysis are presented in Table 1.

Table 1. Chemical soil characteristics.

Treatment	pH	EC	OM	Al	P	S	CEC	Ca	Mg	K	Fe	Cu	Mn	Zn	B
		(Ds m^{-1})	(%)		(mg kg^{-1})			(cmol kg^{-1})					(mg kg^{-1})		
204	5.17	2.27	20.93	0.39	44.25	98.14	18.56	13.90	2.29	1.52	96.73	4.94	11.51	78.62	2.31
278	5.68	0.83	21.87	*	33.96	51.55	16.42	11.65	3.01	1.62	138.53	4.79	4.91	202.12	3.61
333	5.15	1.55	21.88	0.58	43.10	71.05	14.94	10.86	2.11	0.90	133.38	5.23	12.77	78.09	1.98
400	5.82	1.49	11.18	*	73.76	43.63	19.17	14.89	2.78	1.33	152.71	6.09	7.60	64.26	1.77
625	4.94	2.04	23.86	1.94	99.28	174.86	12.38	5.94	1.14	2.67	815.03	9.77	7.69	78.78	4.93
816	5.14	1.06	19.56	0.85	46.65	69.05	7.78	4.61	1.05	0.82	143.77	7.01	5.34	32.67	2.31

*Not available

Mineral leaf content

Four leaves per tree were selected for the analysis of leaf tissue, one at each cardinal point. The fifth leaf of the last growth flow was collected after each harvest (2019M, 2020S, 2020M, 2021S, 2021M). The leaf was mature but not senescent, without fruiting, healthy (without physical or chemical damage or affected by pests or diseases), and older than three months, according to Maldonado (2002). The leaves were washed with distilled water and dried at 60 °C for 48 h in an oven with forced air circulation, Memmert UL 80 (Memmert GmbH + Co. KG, Büchenbach, Germany), or until a constant weight was reached. Next, they were milled, and placed in paper bags, and the total contents of N (EPA method 351.3) where determinated (USEPA 1993), P, K, Ca, Mg, Cl, S, Fe, Cu, Mn, Zn (inductively coupled plasma atomic emission spectrometer iCAP 7000 Plus (Thermo Scientific, Waltham, MA)), and B according to modified NTC 5404 (ICONTEC 2011).

Nutritional balance index

To determine the nutritional balance index (NBI), Salazar-García and Lazcano-Ferrat (2001) adjusted the Kenworthy methodology for the *cv*. Hass avocado was used. The nutrient leaf analysis results were used to determine the balance index (B) for each mineral element, according to Equations (1) and (2).

Equation 1. Suppose the value reported in the laboratory (X) was less than the standard value:

$$P = (X/S)100 \quad I = (100-P)\,(CV/100) \quad B = P + I \qquad (1)$$

Equation 2. If the value reported in the laboratory (X) was greater than the standard value:

$$P = (X/S)100 \quad I = (P-100)\,(CV/100) \quad B = P + I \qquad (2)$$

where, S = standard value, I = influence of the variation, P = percentage of the standard, CV = coefficient of variation and B = balance index. Standard values (S) and coefficients of variation (CV) were used according to Salazar-García and Lazcano-Ferrat (2001).

Mineral fruit composition

To evaluate the concentration of nutrients, 75 fruits were randomly taken per plant distance, and each harvest with approximately 24% of dry material. Subsequently, the fruit structures were individualized, removing the peel, pulp, seed coat, and seed. All the fruit components were dried until a constant weight was reached in forced air ovens at 60 °C. After drying, all fruit components were milled to determine N (EPA method 351.3) (USEPA 1993), P, K, Ca, Mg, Cl, S, Fe, Cu, Mn, Zn (inductively coupled plasma atomic emission spectrometer iCAP 7000 Plus (Thermo Scientific, Waltham, MA)), and B according to modified NTC 5404 (ICONTEC 2011), by treatment and harvest in each location.

Fruit fatty acid and vitamin E profile

Five avocado fruits were taken per tree (180 per treatment) for each harvest season (2019M and 2020S) to quantify the fatty acid content. For each fruit, the mesocarp (pulp) was removed, homogenized, and lyophilized to determine the content (grams of fatty acid per 100 grams of fresh pulp) of oleic, palmitoleic, palmitic, linoleic, eladic, stearic, and fatty saturated acid. A gas chromatograph coupled to a mass spectrometer (Agilent 7890/MSD 5975C) was used, equipped with a capillary column (ZB-5 Zebron of low polarity) to separate the compounds of interest.

Vitamin E content (g α-tocopherol/100 g fresh pulp) was determined from five avocado fruits per tree (180 fruit per treatment) for each harvest season (2019M and 2020S). The mesocarp (pulp) was removed, homogenized, and frozen for each fruit. A gas chromatograph coupled to a mass spectrometer (Agilent 7890/MSD 5975C) was used before derivatization using BSTFA.

Fruit caliber

A random selection of 75 fruits per tree was each harvested. It was weighted and individually characterized by caliber according to established by the FAO in the CODEX STAN 197-1995 Revision (Table 2) for export sizes (FAO 2011).

Table 2. Fruit quality of avocado cv. Hass is characterized by weight and size for export according to CODEX STAN 197-1995.

Caliber	Fruit weight (g)		Caliber	Fruit weight (g)	
	Minimum	Maximum		Minimum	Maximum
Discard	0	80	24	170.1	181
Industrial	80.1	94	22	181.1	200
32	94.1	135	20	200.1	217
30	135.1	149	18	217.1	249
28	149.1	160	16	249.1	284
26	160.1	170	14	284.1	600

(FAO 2011).

Statistical analysis

The statistical analysis was performed using the agricolae package in the R project statistical program (R Core Team 2021). A two-way ANOVA was carried out for the plant density factor (204, 278, 333, 400, 625, and 816 trees ha^{-1}) and the harvest season factor (2019M, 2020S, 2020M, 2021S, and 2021M). Differences between means were evaluated through analysis of variance, followed by Tukey's HSD mean comparison test, with a probability greater than 95%. A Pearson correlation analysis was used to examine the relationship among the content of all nutrients in the fruit and leaves of avocado.

RESULTS AND DISCUSSION

Leaf mineral composition

Table 3 shows the nutritional composition of avocado tree leaves, where there were significant differences due to plant density (*P*<0.001) and harvest time (*P*<0.001). The 333 and 400 trees ha^{-1} plant densities presented the highest leaf concentrations of N, P, Ca, Mg, S, Zn, and B. For K and Fe, higher concentrations were observed in the density of 625 trees ha^{-1}, and Mn was present in higher concentrations in the density of 204 trees ha^{-1}. On the contrary, the highest and lowest densities (204 and 816 trees ha^{-1}) reached the lowest concentrations of the evaluated nutrients. The 2019M harvest presented the highest levels of nutrients for N, Ca, Mg, S, Fe, Mn, and Zn; the other nutrients do not show marked significant differences at a particular plant density (Table 3).

Table 3. Effect of plant density and harvest season on *cv.* Hass avocado leaf mineral composition.

Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	B
	(%)						(mg kg^{-1})			
Plant density*/*P* values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
204	2.18^{b}	0.11^{b}	0.97^{c}	1.42^{b}	0.35^{b}	0.19^{c}	60.82^{e}	166.31^{a}	37.82^{b}	45.1^{2b}
278	2.09^{cd}	0.09^{c}	0.95^{cd}	1.36^{bc}	0.33^{b}	0.16^{e}	104.75^{b}	105.92^{bc}	22.86^{c}	24.53^{e}
333	2.25^{b}	0.12^{b}	0.87^{d}	2.01^{a}	0.40^{a}	0.24^{b}	74.98^{d}	124.97^{b}	28.95^{c}	30.90^{d}
400	2.36^{a}	0.14^{a}	1.06^{b}	2.03^{a}	0.40^{a}	0.28^{a}	72.02^{d}	118.17^{bc}	49.91^{a}	50.76^{a}
625	2.17^{b}	0.09^{c}	1.14^{a}	1.28^{c}	0.33^{b}	0.19^{cd}	117.11^{a}	103.77^{c}	25.30^{c}	36.93^{c}
816	2.03^{c}	0.11^{b}	1.01^{bc}	1.45^{b}	0.39^{a}	0.18^{de}	85.43^{c}	38.75^{d}	22.69^{c}	26.78^{e}
Year/*P* values**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2019M	2.23^{ab}	0.10^{b}	1.01^{b}	2.01^{a}	0.43^{a}	0.28^{a}	122.34^{a}	164.96^{a}	39.09^{a}	30.56^{c}
2020S	2.08^{d}	0.10^{b}	1.00^{b}	1.40^{d}	0.35^{b}	0.19^{b}	78.87^{c}	92.19^{c}	27.27^{bc}	46.71^{a}
2020M	2.28^{a}	0.12^{a}	0.90^{c}	1.50^{c}	0.33^{b}	0.18^{b}	87.22^{b}	92.94^{c}	26.77^{c}	36.87^{b}
2021S	2.16^{bc}	0.11^{a}	1.08^{a}	1.34^{d}	0.34^{b}	0.19^{b}	58.04^{d}	72.22^{d}	30.33^{bc}	37.31^{b}
2021M	2.15^{cd}	0.12^{a}	1.02^{ab}	1.71^{b}	0.37^{b}	0.19^{b}	82.79^{bc}	125.94^{b}	32.81^{b}	27.72^{d}
Mean	2.18	0.11	1.00	1.59	0.36	0.21	85.85	109.65	31.25	35.83

*Trees ha^{-1} **Main harvesting (M). Secondary harvesting (S). Values with similar letters for each nutrient within location or rootstock/scion relation are significantly different (HSD, *P*<0.05).

In addition to the foliar nutrient diagnosis status and the amount of nutrients removed, the assimilation dynamics of the different nutrients during fruit development are important to precise fertilization management decisions (Silber et al. 2018; Salazar-García et al. 2019). Maldonado-Torres et al. (2007) set the nutritional standards for the *cv.* Hass avocado for a yield greater than 20 t ha^{-1}. Therefore, according to the results, the nutritional concentrations of N, K, Ca, Fe, Mn, and Zn were between optimum and high levels, while P was at low levels, and Mg and B were at low concentrations. Maldonado-Torres et al. (2007) stated that the low level of P determined in these soils (Andosols) might be associated with the fixing effect of allophane, which has sometimes retained up to 2,000 mg kg^{-1}. Similar results were found by Tamayo-Vélez et al. (2022), who evaluated the leaf nutritional content in avocado trees in the high Andean region in Colombia.

According to Salazar-García and Lazcano-Ferrat (2003), Maldonado-Torres et al. (2007) and Campos and Calderón (2015) the recommended reference levels of nutrients in leaves for Hass avocado vary for N (1.9 – 2.31%), P (0.11 – 0.18%), K (0.5 – 1.4%), Ca (1.0 – 3.0%), Mg (0.3 - 0.8%), Fe (85 – 114 mg kg^{-1}), B (30 - 240 mg kg^{-1}), Zn (20 – 150 mg kg^{-1}) and Mn (30 – 500 mg kg^{-1}). From these reference values and according to the mineral composition of the leaf, only values below the reference were observed for P (278 and 625 trees ha^{-1}), which indicates that despite having observed significant differences in the concentration of nutrients in the leaf in response to the planting densities, in general the observed values were within the reference ranges for an optimal condition for the Hass cultivar water (Maldonado-Torres et al. 2007).

Nutritional balance index

There were significant differences in plant density in the nutritional balance index (NBi) for N, Ca, Mg, S, Fe, Mn, Zn, and B. While NBi for P and K did not present significant differences (P>0.05) for any of the plant densities evaluated. Similarly, the 2019M harvest season showed the highest NBi for all mineral nutrients except boron (Table 4).

Balanced and timely nutrition is essential to ensure the yield and quality of avocados. The avocado cultivar, the management practices, and the environmental conditions where it is cultivated influence the concentration of nutrients in the leaves and fruits (Salazar-García et al. 2019). Hence, in assessing the nutritional status of plants, Manzoor et al. (2022) recommend using the nutritional balance index (NBI) to seek the plants' nutrient balance. Leaf nutritional content allows the avocado leaves, according to the nutrient balance index proposed by Kenworthy (1973), as scarce (15-49%), below normal (>49-83%), normal (>83-117%), above normal (>117-151%), and excess (>151-185%). According to our results, although there were differences between plant densities, the N, P, K, Ca, and Fe generally had normal balances, while Mg, S, and Mn were below normal, indicating possible nutritional deficiency without symptoms being observed. Similar results were reported by Tamayo-Vélez et al. (2022), who evaluated the effect of rootstock/scion compatibility in the same regions. These authors found that the N, P, Ca, Fe, and Zn showed normal NBi, while K, Mg, S, Mn, and B were below normal, indicating possible nutritional deficiency without symptoms being observed.

Table 4. Nutritional balance index (NBi) for the avocado *cv.* Hass leaf for different plant densities and harvest seasons.

Treatment	NB	PB	KB	CaB	MgB	SB	FeB	MnB	ZnB	BB
Plant density*/ *P value*s	<0.001	0.07	0.403	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
204	94^{bc}	114	114	81^{b}	66^{b}	54^{c}	80^{d}	82^{a}	154^{b}	80^{b}
278	91^{cd}	68	74	78^{bc}	63^{b}	47^{e}	128^{b}	66^{b}	90^{c}	66^{e}
333	97^{b}	85	69	111^{a}	73^{a}	66^{b}	90^{cd}	73^{b}	119^{c}	70^{d}
400	101^{a}	99	81	113^{a}	73^{a}	73^{a}	87^{d}	69^{b}	213^{a}	84^{a}
625	93^{bc}	68	86	75^{c}	64^{b}	52^{cd}	146^{a}	65^{b}	100^{c}	74^{c}
816	88^{d}	81	78	82^{b}	72^{a}	50^{de}	102^{c}	49^{c}	92^{c}	67^{e}
Year/*P* values**	<0.001	0.644	0.228	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2019M	96^{ab}	73	78	112^{a}	78^{a}	74^{a}	154^{a}	83^{a}	167^{a}	70^{c}
2020S	90^{c}	97	77	80^{cd}	66^{b}	53^{b}	96^{c}	62^{c}	104^{c}	81^{a}
2020M	98^{a}	88	71	84^{c}	69^{b}	52^{b}	104^{c}	63^{c}	107^{c}	74^{b}
2021S	93^{bc}	83	114	78^{d}	66^{b}	54^{b}	96^{b}	57^{c}	127^{bc}	75^{b}
2021M	93^{bc}	88	79	95^{b}	69^{b}	53^{b}	78^{c}	71^{b}	135^{b}	68^{d}
Mean	94	86	84	90	70	57	106	67	128	74

* Trees ha^{-1} **Main harvesting (M). Secondary harvesting (S). Values with similar letters for each nutrient within location or rootstock/scion relation are significantly different (HSD, P<0.05).

Plant density is known to affect plant traits and growth above ground significantly. Nevertheless, understanding the effects of sowing density on fruit and leaf nutrient accumulation is essential in determining an adequate fertilization program to guarantee acceptable yield and fruit quality (Hecht et al. 2019). More plants at higher plant densities require and take up more nutrients. This does not necessarily occur proportionally due to competition, which can be asymmetric when plants of different sizes compete. Instead, the competition might trigger similar root growth and canopy plasticity responses as low resource availability significantly changes the root and canopy system architecture and functioning (Hecht et al. 2019; Tun et al. 2018; Cano-Gallego et al. 2023) and soil elements availability (Reddy et al. 2014).

Fruit mineral composition: The plant density significantly does not affect the nutrients in the avocado fruit for the minerals K, Ca, S, and Fe. Conversely, the contents of N, P, Mg, Na Cu, Mn, Zn, and B were significantly affected by plant density. However, a homogeneous behavior was not observed in the variation of this variable; in general, the plant densities of 333 and 400 trees ha^{-1} presented high contents of all nutrients in fruit, except for Cu, whose contents were medium (Table 5). A similar result was reported: nitrite content in the fruit is also the lowest in the low-density treatment in cucumber (Ding et al. 2022), and a positive relation was found between leaf N to pulp N concentrations (Arpaia et al. 1996).

Regarding the nutritional composition in each fruit tissue, the highest contents of all the nutrients were observed in the seed

Table 5. Effect of plant density on *cv.* Hass avocado fruit mineral composition assessment in Colombia. Values are averages of 3 years (2019-2021).

Treatment	N	P	K	Ca	Mg	Na
	(%)					
Plant density*/*P* values	<0.001	<0.001	<0.001	0.121	<0.01	<0.001
204	0.8895^{a}	0.0805^{bc}	1.3450^{a}	0.1196	0.1087^{b}	0.0049^{bc}
278	0.7693^{c}	0.0662^{c}	1.2425^{a}	0.1510	0.1049^{b}	0.0072^{ab}
333	0.8730^{ab}	0.1111^{a}	1.3300^{a}	0.1397	0.1150^{b}	0.0037^{bc}
400	0.8982^{a}	0.1079^{a}	1.3589^{a}	0.1168	0.2116^{a}	0.0015^{c}
625	0.8251^{abc}	0.0618^{c}	1.3579^{a}	0.1368	0.0987^{b}	0.0108^{a}
816	0.8081^{bc}	0.1009^{ab}	1.3241^{a}	0.1426	0.1156^{b}	0.0031^{bc}
Fruit tissue/*P* values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05
Peel	0.835^{c}	0.083^{b}	1.115^{c}	0.123^{b}	0.095^{b}	0.004^{ab}
Pulp	0.917^{bc}	0.105^{a}	1.726^{a}	0.106^{b}	0.108^{b}	0.008^{a}
Seed coat	0.973^{a}	0.086^{b}	1.487^{b}	0.255^{a}	0.255^{a}	0.005^{ab}
Seed	0.650^{d}	0.078^{b}	0.978^{d}	0.052^{c}	0.045^{b}	0.004^{ab}
Treatment	**S**	**Fe**	**Cu**	**Mn**	**Zn**	**B**
	(%)	(mg kg^{-1})				
Plant density*/*P* values	0.0997	<0.01	<0.001	<0.001	<0.001	<0.001
204	0.072	28.840^{ab}	12.194^{a}	19.344^{a}	20.055^{ab}	65.922^{a}
278	0.070	28.777^{ab}	4.228^{cd}	18.270^{a}	16.459^{c}	40.065^{c}
333	0.072	23.929^{b}	6.313^{bc}	17.313^{a}	20.205^{ab}	53.865^{b}
400	0.080	26.087^{ab}	6.867^{b}	16.893^{a}	21.881^{a}	63.952^{a}
625	0.067	31.063^{a}	2.640^{d}	15.496^{b}	17.784^{bc}	63.553^{a}
816	0.063	27.594^{b}	3.605^{d}	11.448^{b}	16.268^{c}	40.903^{c}
Fruit tissue/*P* values	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Peel	0.054^{b}	32.256^{b}	8.304^{a}	10.424^{b}	18.269^{b}	54.254^{b}
Pulp	0.090^{a}	24.442^{c}	4.469^{b}	7.072^{bc}	15.905^{c}	50.048^{b}
Seed coat	0.088^{a}	39.548^{a}	7.834^{a}	42.567^{a}	32.387^{a}	84.661^{a}
Seed	0.051^{b}	14.613^{d}	3.291^{b}	5.780^{c}	8.541^{d}	29.877^{c}

*Trees ha^{-1} ** Values with similar letters for each nutrient within location or rootstock/scion relation are significantly different (HSD, $P<0.05$).

coat and pulp. In contrast, the peel and seed only presented higher values than the rest of the tissue in Na levels. Similar results were reported by Tamayo-Vélez et al. (2022), who found, in general, the highest nutrient contents in the seed coat. The seed coat provides an interface between the embryo and the external environment, can explain altered processes in fruits during embryogenesis, dormancy, and germination, and has a role in determining seed size (Haughn and Chaudhury 2005).

The concentration of elements in avocado fruits was in decreasing order of K>N>Ca>Mg>P>S>B>Fe>Zn>Mn>Cu. In this regard, Reddy et al. (2014) found a similar element concentration in avocado *cv*. Hass and Fuerte (Mg>Ca>Zn>Fe>Mn>Cu). Since the mesocarp is the edible part of the avocado fruit, its quality at consumption maturity usually identifies whether adequate orchard management was carried out (Salazar-García et al. 2019). It suggested that avocado fruits are nutritional as a good dietary source of the micronutrients Cu and Mn, contributing 75 and 34% towards dietary reference intake for these elements by avocado fruit consumption (Reddy et al. 2014). Mesocarp concentrations of both N and Ca are relevant for postharvest fruit quality (Salazar-García et al. 2019). Fruit from trees high in N showed increased susceptibility to chilling injury and a shorter ripening time (Kruger et al. 2016). Otherwise, high Ca levels have decreased cold-induced disorders (Salazar-García et al. 2019).

Some antagonistic and synergistic relationship between the soil exchangeable minerals and mineral concentration in avocado *cv*. Hass fruit has been reported by Reddy et al. (2014). Soil Al excess reduces *cv*. Hass fruit Cu (-0.8) and Fe (-0.9), Fe excess reduces Cu (-0.9) and Zn (-0.7), Ca excess reduces Mn (-0.7), and Mg excess reduces Mg (-0.8). On the other hand, the synergistic effects include soil Al excess increase in the Mn (0.8) and Cu (0.9) fruit concentration, Soil Mg excess increase in Mn (1.0) and Cu (0.9) fruit concentration, and soil Ca excess increase in Mn (0.7), Ca (0.7) and Cu (0.7) fruit concentration. The local soil presented strongly acid soils with high organic matter content, high and very high Ca, Mg with medium to low ranges, and high K. Minor elements are reported to have very high contents of iron, medium to high for manganese and zinc, and medium to low for copper and boron (Tamayo-Vélez et al. 2022). Ding et al. (2022) demonstrated that changing plant density has significantly improved the quality of the cucumber fruit.

In the case of avocado *cv*. Hass, it has been observed that increasing the N concentration in the leaf leads to a higher N content in the pulp (Tamayo et al. 2018). This finding was further confirmed in this study, where the highest leaf and fruit N concentrations were observed at intermediate plant densities (333 and 400 trees ha^{-1}). This suggests that plant density can play a significant role in determining fruit quality, with intermediate plant densities leading to higher N concentrations in the fruit.

Correlation matrix

The correlation matrix shows that some elements are associated with others in avocado leaves and fruits (Figure 1). The conventional approach to interpreting a correlation coefficient correlation selected was a very strong correlation (greater than 0.900), strong correlation (between 0.700 and 0.899), moderate correlation (between 0.400 and 0.699), and weak correlation (between 0 and 0.399) (Schober et al. 2018). S is positive and strongly associated with Ca in leaves and moderately associated with Mg, Zn, and N. Mg is moderately associated with Ca. On the other hand, P and Fe show a negative moderate association. In addition, some elements, mainly Fe and K, show no or minimal correlation with others (Figure 1A). In fruit, Zn is positive and strongly associated with Mn and B and moderately associated with Fe, Ca, and N. B is moderately associated with N, Mn, and K. Conversely, no negative association was observed, and P shows no or minimal correlation with other (Figure 1B). Lazare et al. (2020) reported significant correlations in most nutrients with the others in *cv*. Hass leaves with the others. The strongest correlations (more than 0.7) were between Mg and Ca, P and Cu, Mg and Mn, N and P, and Mg and N.

Silber et al. (2018) demonstrated high Zn concentration in the productive organs of avocados probably because Zn is transported in the phloem, unlike the other elements that are transported in the xylem. Zn is involved in diverse plant functions such as photosynthesis, sucrose and starch formation, protein metabolism, membrane integrity, auxin metabolism, flowering, and seed production. Zn shows a significant correlation with N, P, and B in leaves, while in fruits, with Cu, Fe, and S (Figure 1).

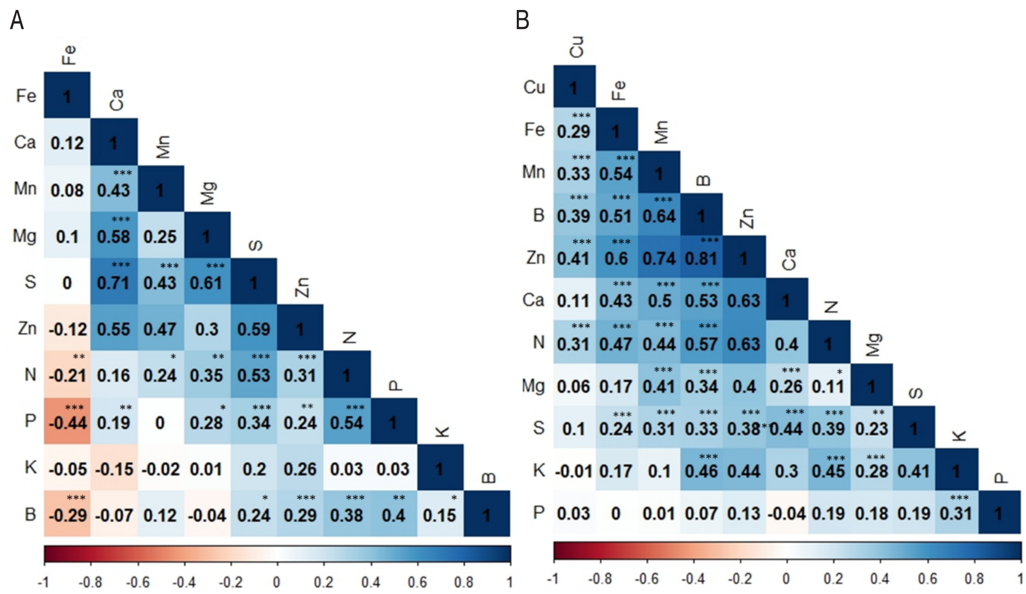

Figure 1. Pearson pairwise correlation matrix among all the nutrient contents in leaves (A) and fruit (B) of avocado *cv.* Hass. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Fatty acid composition of avocado fruit mesocarp

Figure 2 summarizes the contents of fatty acids present in the pulp of the fruits for the plant densities (Figure 2A) and harvest seasons (2019M and 2020S) (Figure 2B). The fruit harvested from trees in high densities presented the highest concentrations (g 100 g^{-1}) of Palmitoleic acid (3.01), Palmitic acid (7.15), Elaidic acid (1.61), and saturated fatty acid (7.38). On the contrary, the plant density of 333 and 400 trees ha^{-1} had the lowest contents for this same group of acids (Palmitoleic acid - 0.55 g; Palmitic acid - 1.63 g; Linoleic acid - 1.11 g; Oleic acid – 2.63 g; Elaidic acid - 0.44 g; and saturated fatty acid - 1.73 g). Regarding the harvest season, in the 2020S, higher content of Palmitoleic acid (2 g), Palmitic acid (5.38 g), Linolenic acid (1.86 g), Elaidic acid (1.17 g), Stearic acid (0.19 g), and saturated fatty acid (5.57 g) were recorded. The 2019M harvest only presented higher values for Oleic acid (2.63 g).

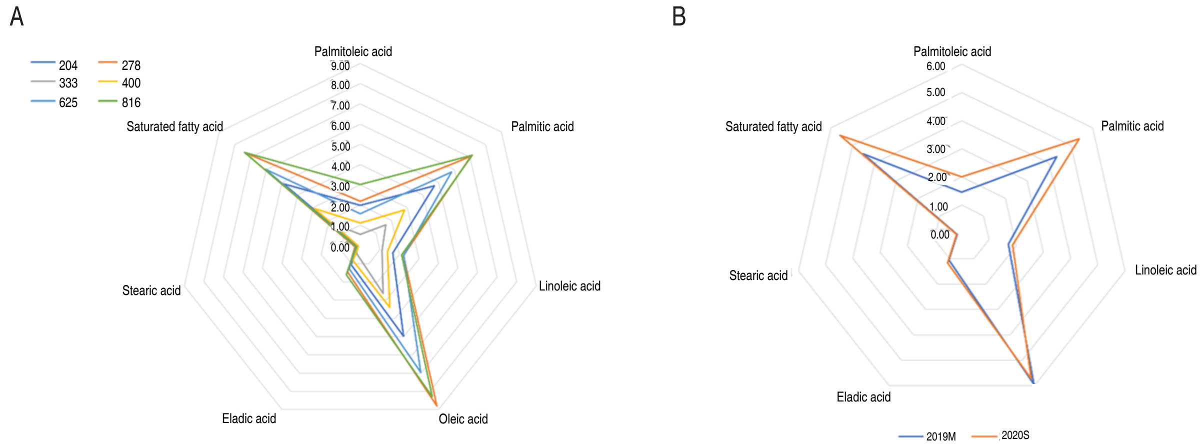

Figure 2. Fatty acid (g 100 g^{-1}) composition of avocado fruit mesocarp for A) plan density (tree ha^{-1}) and B) harvest season (2019M and 2020S). *Main Harvesting (M). Secondary Harvesting – Mitaca (S).

Vitamin E content in avocado fruit mesocarp

Vitamin E content is presented descriptively in Figure 3. The concentrations of vitamin E (mg α-tocopherol 100 g^{-1}) were similar in the plant densities of 204 (1.42), 278 (1.45), 400 (1.36), and 625 (1.415) trees ha^{-1}. The lowest values (0.905) were reached in the density of 333 trees ha^{-1}. Similar to what was observed for fatty acid concentration, a lower fatty acid level was observed at this plant density. Regarding harvest time, vitamin E contents were observed for 2019M (1.24) and 2020S (1.35). It has been reported that vitamin A contents rank for 1.78–3.5 mg α-tocopherol 100 g^{-1} (Jimenez et al. 2020). Nonetheless, this study's overall vitamin E content was low (0.90–1.45).

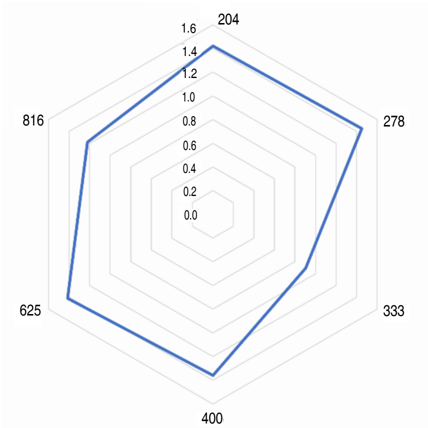

Figure 3. Vitamin E content (mg α-tocopherol 100 g^{-1}) in avocado fruit mesocarp for plant density (tree ha^{-1}).

Avocado fruit calibers

For many years, the profitability of avocado production was measured in terms of total fruit yield. However, this parameter has lost importance due to the avocado market. Harvest time, size, and fruit quality (external and internal) are considered the main factors for successful avocado marketing (Salazar-García and Lazcano-Ferrat 2001). However, the percentage of avocado fruits by size showed no significant differences due to plant distances. The harvest factor significantly affected most of the percentage proportions of each caliber in the avocado fruit. Despite the effect of the harvest season, a homogeneous behavior was not observed in the variation of this variable (Table 6).

Related to crop yield, it is reported that planting density affects avocado fruit yield. Cano-Gallego et al. (2023) found that yield per tree and number of avocado fruits per tree are negatively affected by the increase in planting densities. In addition, fruit quality parameters show better results at intermediate planting densities of 333 and 400 trees per hectare. The intermediate densities of 333 and 400 trees produced the highest fruits per tree (323) and the highest fruit yield (19 t ha^{-1}).

Table 6. Percentage proportions of the weight of each caliber in avocado trees *cv.* Hass for five plant densities during five harvests (2020M, 2020P, 2021M, and 2021P).

Treatment	C14	C16	C18	C20	C22	C24
Plant density*/*P values*	0.19	<0.05	<0.01	<0.01	<0.001	0.11
204	1.05	3.17^{ab}	7.84^{a}	7.59^{ab}	14.91^{a}	6.75
278	0.34	1.45^{b}	7.84^{b}	5.97^{b}	9.79^{b}	7.33
333	1.55	4.02^{a}	8.36^{a}	7.32^{ab}	10.91^{ab}	7.01
400	0.89	2.85^{ab}	8.65^{a}	10.58^{a}	14.43^{a}	8.66
625	1.01	2.66^{ab}	7.99^{a}	6.86^{b}	14.89^{a}	7.40
816	1.17	2.03^{ab}	6.12^{ab}	5.64^{b}	12.63^{ab}	8.57

(Table 6)

Treatment	C14	C16	C18	C20	C22	C24
Year/*P values***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2019M	2.34^{a}	6.11^{a}	12.21^{a}	9.93^{a}	14.80^{a}	7.27^{ab}
2020S	0.89	1.75^{b}	5.53^{b}	7.63^{ab}	15.61^{a}	8.11^{ab}
2020M	0.48^{b}	2.04^{b}	5.86^{b}	6.33^{b}	10.41^{b}	6.52^{b}
2021S	0.89^{b}	1.88^{b}	6.86^{b}	6.84^{b}	12.65^{ab}	9.09^{a}
2021M	0.41^{b}	1.70^{b}	5.24^{b}	5.91^{b}	11.18^{b}	7.12^{ab}
Treatment	**C26**	**C28**	**C30**	**C32**	**DC**	**IC**
Plant density*/*P values*	0.089	0.881	0.123	<0.001	<0.05	<0.05
204	7.01	9.59	10.60	21.94^{b}	5.36^{ab}	4.18^{ab}
278	6.85	8.44	12.15	32.15^{a}	5.75^{a}	5.92^{a}
333	6.56	9.87	10.30	24.26^{ab}	5.23^{ab}	4.60^{ab}
400	7.85	9.72	10.16	18.80^{b}	3.15^{b}	4.25^{ab}
625	8.27	9.42	11.92	22.23^{b}	3.74^{ab}	3.60^{ba}
816	8.27	9.48	10.88	26.79^{ab}	3.73^{ab}	4.69^{b}
Year/*P values***	0.557	<0.001	<0.001	<0.001	<0.001	<0.001
2019M	7.07	7.89^{ba}	7.85^{b}	18.56^{b}	2.89^{c}	3.07^{b}
2020S	7.00	9.50^{ab}	11.94^{a}	23.94^{ab}	3.89^{bc}	4.21^{ab}
2020M	7.55	8.63^{ab}	11.42^{a}	28.73^{a}	6.21^{a}	5.82^{a}
2021S	7.74	11.65^{a}	11.42^{a}	23.48^{ab}	3.44^{c}	4.09^{ab}
2021M	7.98	11.65^{ab}	12.39^{a}	27.11^{a}	6.03^{ab}	5.51^{a}

*Trees ha^{-1} **Main Harvesting (M). Secondary Harvesting "Mitaca" (S). *** Values with similar letters for each nutrient within location or rootstock/scion relation are significantly different (HSD, P<0.05).

CONCLUSION

The planting density significantly affected the leaf and fruit nutritional composition except for Ca; thus, the nutritional balance index (NBi) for N, Ca, Mg, S, Fe, Mn, Zn, and B. The highest nutrient concentrations in the leaves and fruits of Hass avocado and the lowest saturated fatty acid contents in fruits were obtained at the intermediate plant densities of 333 and 400 trees per hectare. On the other hand, the percentage of avocado fruits by size showed no significant differences due to plant distances. There were intermediate planting densities (333 and 400 trees ha^{-1}). The dynamics of the accumulation of nutrients in avocado leaves and fruit through the different leaf ontogeny and fruit development are recommended to determine if planting density through the Hass avocado phenology is related to the nutritional contents of the leaf and fruit.

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CONFLICT OF INTERESTS

The manuscript was prepared and reviewed with the participation of the authors, who declared that there exists no conflict of interest that puts at risk the validity of the presented results.

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Impact of nutrient omission technique on the formation of a simple hybrid of corn (*Zea mays* L.)

UNIVERSIDAD NACIONAL DE COLOMBIA

Impacto de la técnica de omisión de nutrientes en la formación de un híbrido simple de maíz (*Zea mays* L.)

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ABSTRACT

Due to the scarcity of information in Ecuador on the effect of nutrient omission in the fertilization of parents for the formation of a new maize hybrid, this research aimed to evaluate the impact of nutrient omission on the formation of a maize hybrid and its seed yield. The study was carried out at the Estación Experimental Tropical Pichilingue (EETP) del Instituto Nacional de Investigaciones Agropecuarias (INIAP), Los Ríos province, Ecuador. Six omission treatments were established -N, -P, -K, -Mg, -S, -B, complete fertilization (N, P, K, Mg, S, B), a treatment based on the application of a farmer and control. A randomized complete block design was used and the averages were differentiated using Tukey's test. The variables evaluated were: plant height (m), ear height (m), days to flowering, cob length (cm), cob diameter (mm), 100-seed weight (g), percentage of rotten ears, stem diameter (mm), stem and root lodging, chlorophyll levels, yield, partial productivity factor, agronomic efficiency and nutrient harvest index. The results obtained showed that the highest yield was obtained with the omission of the B (2,093 kg ha^{-1}) fertilization plan causing a decrease of up to 42.02% in the partial productivity factor (PPF) and 425.14% of agronomic efficiency (AE) of the studied elements. Since the nutritional priorities of the parents of the corn hybrid had the sequence Mg>N>K=P>S>>B.

Keywords:
Crop management
Fertilizers
Plant nutrition
Production
Soil

RESUMEN

Debido a la escasa información en el Ecuador sobre el efecto de la omisión de nutrientes en la fertilización de los progenitores para la formación de un nuevo híbrido de maíz, el objetivo de esta investigación fue evaluar el impacto de la omisión de nutrientes en la formación de un híbrido de maíz y su rendimiento de semilla. El estudio se realizó en la Estación Experimental Tropical Pichilingue (EETP) del Instituto Nacional de Investigaciones Agropecuarias (INIAP), provincia de Los Ríos, Ecuador. Se establecieron seis tratamientos de omisión de -N, -P, -K, -Mg, -S, -B, fertilización completa (N, P, K, Mg, S, B), un tratamiento basado en la aplicación de un agricultor (N, P, K) y un testigo. Se utilizó un diseño de bloques completos al azar y los promedios diferenciados mediante la prueba de Tukey. Las variables evaluadas fueron: altura de planta (m), altura de mazorca (m), días de floración, longitud de mazorca (cm), diámetro de mazorca (mm), peso de 100 semillas (g), porcentaje de mazorcas podridas, diámetro de tallo (mm), acame de tallo y de raíz; niveles de clorofila, rendimiento, factor parcial de productividad, eficiencia agronómica e índice de cosecha de nutrientes. Los resultados obtenidos mostraron que el mayor rendimiento se consiguió con la omisión de B (2.093 kg ha^{-1}), esto debido a que su presencia en el plan de fertilización provoca disminución de hasta el 42,02% en el factor parcial de productividad (FPP) y 425,14% de eficiencia agronómica (EA) de los elementos estudiados. Siendo que las prioridades de nutrición de los parentales del híbrido de maíz, tuvieron la secuencia Mg>N>K=P>S>>B.

Palabras clave:
Manejo de cultivos
Fertilizantes
Nutrición vegetal
Producción
Suelo

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Worldwide, corn (*Zea mays* L.) is one of the most versatile crops and adapts to different environmental conditions. Its uses range from human food, to animal feed and as raw material for a large number of industrial products (Ayyar et al. 2019). In Ecuador, corn production and productivity in the Coast and Amazon regions have increased in recent years due to greater technification, which has made it possible to reduce corn imports. These increases were made possible by innovations in genetic improvement, with the development of varieties and hybrids with high yield potential (Caviedes-Cepeda et al. 2022).

Hybrid corn is a unique and highly specialized crop, its production requires time, money and experience. Obtaining hybrid corn seeds involves crossing two inbred lines, one of which is called "male", which is responsible for the production of pollen and 'female' the plant that creates the hybrid seed, the purpose is to create a variety that shows some important characteristic, for example, resistance to pathogen (Mirsam et al. 2021).

The corn program of the Estación Experimental Tropical Pichilingue (EETP) del Instituto Nacional de Investigaciones Agropecuarias (INIAP), is constantly seeking to generate new promising genetic materials to improve the economic income of producers, mainly small and medium-sized ones (Zambrano et al. 2018). However, the optimal yield of genetic materials in corn can be compromised by inadequate fertilization, as sometimes the nutrient reserves in the soil are not sufficient to meet the crop's demands. Therefore, balanced fertilization is necessary to achieve quality corn production. The incorporation of nutrients such as magnesium, phosphorus, nitrogen and potassium in adequate form is necessary for the growth, development and yield of the crop, considering that potassium improves the productivity of irrigation water (Ul-Allah et al. 2020).

The nutrient omission or missing element technique allows for determining the impact of not applying a specific nutrient on crop yields. This method involves supplementing the soil with nutrients while excluding one specific nutrient, while maintaining adequate levels of the others. This helps identify which nutrient has the greatest effect on both crop yield and the absorption of other nutrients (Shankar et al. 2021). Additionally, this technique can be used to evaluate the synergism or antagonism in the absorption of heavy metals, which may affect the safety of the resulting products(Valarezo et al. 2022).

To achieve high conversion efficiency of intercepted radiation into biomass and achieve high yields in corn it is necessary to know the nutritional requirements of the crop and the contribution of the soil to estimate its fertilization needs, research conducted by Hasang et al. (2018) in evaluating maize hybrid seed production with two homozygous lines in response to the missing element technique in maize, determined that the best yield potential, 2,134 kg ha^{-1}, was obtained with the sulfur omission treatment; furthermore, it was estimated that the fertilization priorities were P>N=Mg=K>>Zn>B>>S. It was also found that ear length and diameter were reduced when the K application was omitted. Due to the little information in the country on the effect of nutrient omission in the fertilization of parents for the formation of a new maize hybrid, this research aimed to evaluate the impact of nutrient omission on the formation of a maize hybrid and its seed yield.

MATERIALS AND METHODS

Location of the trial

The study was carried out at the Estación Experimental Tropical Pichilingue (EETP) del Instituto Nacional de Investigaciones Agropecuarias (INIAP), located in the province of Los Ríos, canton Mocache, 5 km from the Quevedo - El Empalme road. It is located at 79°280' W longitude and 01°060' S latitude, at 75 meters above sea level (masl), with an average temperature of 24.5 °C, average annual rainfall of 1,723 mm and relative humidity of 84.33%.

Edaphic characteristics and genetic material.

The edaphic characteristics of the experimental area were determined using the methodologies described by Quezada et al. (2017), which resulted in a pH=5.9, nutrient content of NH_4+ (8 mg kg^{-1}), 34 mg kg^{-1} (P), 0.97 meq 100 mL^{-1} (K), 11 meq 100 mL^{-1} (Ca), 2 meq 100 mL^{-1} (Mg), 7 mg kg^{-1} (S), 6.3 mg kg^{-1} (Zn) and 0.97 mg kg^{-1} (B), 5.4% organic carbon and silt loam texture**.** The trial was conducted during the rainy season of 2019; 36 experimental units were established with a planting density of 62,500 plants per hectare, the seeds of homozygous parents; male (CML-172) and female (L-21-3-1-1-1-1-1-1-1 COM 2), provided by the Program de maize of the EETP of INIAP.

Treatments

The study factor was the omission of nutrients to the progenitors, for this purpose six omission treatments (N, P, K, Mg, S and B), a complete fertilization treatment one based on fertilization by a farmer and an absolute control were evaluated (Table 1), the area of each experimental unit was 24 m^2, with a useful area of 9.6 m^2.

Table 1. Treatments evaluated in the nutrient omission experiment in breeding a new simple hybrid of durum corn (*Zea mays* L.). rainy season.

Treatments	N	P_2O_5	K_2O	S	Mg	B
	Kg ha^{-1}					
-N	-	46	60	44	55	1.5
-P_2O_5	140	-	60	44	55	1.5
-K_2O	140	46	-	44	55	1.5
-S	140	46	60	-	55	1.5
-Mg	140	46	60	44	-	1.5
-B	140	46	60	44	55	-
Complete	140	46	60	44	55	1.5
PFP*	140	46	60	-	-	-
Witness	-		-	-	-	-

***PFP:** Producer's fertilizer plot.

Variables evaluated

The chlorophyll levels were recorded with a portable chlorophyll meter atLEAF chlorophyll meter, USA (atLEAF units) at 25, 30 and 35 days after sowing (DAS). The days to flowering were estimated from the day of sowing until 50% of the panicles and cobs emerged. The morphological variables were plant and ear height (cm), stem diameter (mm) at the first inter-node, percentage of stem lodging in plants with more than 45% inclination at the first inter-node and root in plants with more than 30% inclination from the soil surface, were recorded at 107 DAS. At 116 DAS, ear rot (%), ear diameter and length (mm), and hundred-seed weight (g) were evaluated.

The yield (kg ha^{-1}) was determined using Equation 1, with data on fresh harvested grain weight (FGW), and the percentage of grain moisture (GW) in the fertilized female plants of each treatment and the result was adjusted to 14% desired moisture (DM).

$$\text{Yield (kg ha}^{-1}\text{)} = \frac{\text{FGW} \times (100 - \text{GW})}{(100 - \text{DM})} \times \frac{\text{Density (Plants ha}^{-1}\text{)}}{\text{\# Harvested plants}} \quad (1)$$

The fertilization efficiency was determined using the equations described by Dobermann (2007): partial productivity factor (PPF) is calculated as the ratio between the kilograms of seed produced and the kilograms of the applied element, allowing for the evaluation of a specific nutrient's efficiency in production, agronomic efficiency (AE) is defined as the ratio of the increase in production (kg) to the kg of the applied element, helping to understand how much additional yield is obtained per unit of nutrient and nutrient harvest index (NHI) indicates the proportion of absorbed nutrients that will be allocated to the grain, reflecting the crop's ability to transfer nutrients from the soil to the edible part of the plant.

Crop management

The seeds of the parents were treated with insecticide and fungicide before sowing. The soil was prepared by harrowing. For each experimental unit, two rows of the male parent and four rows of the female parent were planted (4:2). Insect pest control was carried out at 5, 15 and 34 DAS, and at 46 DAS a manual application of insecticide bait was made. Disease control was carried out at 35 DAS, and chemical weed control was applied at planting and post-emergence (manually at 47, 82 and 96 DAS). Fertilization was carried out according to the omission treatments, by continuous jet, in four applications: dividing

N into three applications at 15, 30 and 45 DAS, P at sowing, K at 15 DAS, S and Mg two applications at 15 and 30 DAS and B at 30 DAS, using commercial fertilizers: Urea, Triple Super Phosphate, Potassium Muriate, Ammonium Sulfate, Magnesium Sulfate, Magnesium Nitrate, Borax.

Pollination was assisted at the time of panicle emission. At the emission of the female flower from the female parent, a sheath was placed to avoid fertilization with pollen from other treatments, and each ear was pollinated with pollen from the male parent subjected to the same treatment.

To determine the nutrient content in the tissues, the methodologies used by Carrillo et al. (2019) were employed. At the end of the experiment, two plants (aerial part) were taken from each experimental unit, washed with distilled water, and dried in ovens at 70 °C until a constant weight was achieved. Nitrogen analysis was carried out by the Kjeldahl method (KjeltecTM 8400 TecatorTM Line Fost, China). The other elements were determined by wet digestion (nitric-perchloric acid) and the analyses for K, Ca and Mg were measured by an atomic absorption spectrophotometer (AA 6800) Japan, as well as S, P and B by colorimetry (Spercord 210 Plus, Germany).

Data analysis

Data were analyzed using the Randomized Complete Block Design (RCBD) with four replications and comparisons between means with Tukey's 5% test, using the statistical program InfoStat v2018 (Di Rienzo et al. 2018).

RESULTS AND DISCUSSION

Morphological characteristics of the corn plant due to the effect of treatments of nutrient omission

These results show that, although under the conditions of the experiment it was not possible to find differences in some variables, the skipping treatments significantly influenced the morphology of the maize plants. Complete fertilization favored better growth and greater grain weight, while the omission of nitrogen weakened the stems and roots. This underscores the importance of balanced nutrition to avoid structural problems in the crop.

Table 2 shows the plant height of the different treatments applied, the highest height is presented by the fertilized treatment according to the producer (194 cm), followed by the Mg omission treatment (193 cm), probably because this element is not essential for the development of the plant at height, as it is for photosynthesis, where it is an essential component of the chlorophyll molecule. The lowest height was found in the control treatment at 170 cm, these results coincide with those found by Lucero et al. (2023), who found no significant differences for the plant height variable in a trial with hard yellow corn testing different sources of magnesium and silicon.

In the variable days to male and female flowering, ranging between 62-64 and 60-61 days respectively, this two-day variation indicates that this characteristic of the plant is more influenced by its genetics than by nutrition, at least in the soil evaluated, these results differ from those found by Romero-Cortes et al. (2022), because it was made with other genetic material and 2,426 masl, who report significant differences between the different fertilization schemes evaluated ($P \leq 0.05$) 98.67 and 100.67 days for male flowering; 103.33 and 106.33 days for female flowering, in Kculli Purple maize under different fertilization schemes.

Regarding the variable weight of 100 grains, the treatment that obtained the highest weight per 100 grains was the complete fertilization (27 g), while the lowest value was the treatment where the element obtained the omission of P (21 g), these results coincide with what was stated by Marchiori et al. (2020), who affirms that the application of P_2O_5 increases the weight of corn grains.

The variables insertion height, length and cob diameter, the highest cob height was presented in the treatment with the omission of phosphorus (112 cm) and the lowest height was presented by the treatment with the omission of nitrogen and the control (85 cm), these differ from those obtained by Vera et al. (2020) where the effect of three forms of fertilization via edaphic fertilization was evaluated in corn variety DAS 3383, where the ear at 70 days showed significant differences (P<0.05), being the treatment where the conventional fertilization plan was applied the one that showed the highest average with 89.05 cm.

With respect to cob length, there are no differences, probably due to lines with a high degree of homozygosity, used for seed multiplication (Gutiérrez et al. 2016), the values among treatments are very similar, with the lowest values (14 cm) observed in the control treatment and in the omission of B, the highest values are observed in the omission of K and Mg, with 16 cm, these values differ from those obtained by Romero-Cortes et al. (2022), who report significant differences between the different fertilization schemes evaluated for ear length ($P \leq 0.05$), with the best results observed in the treatments where Chemical fertilizer + Bioinoculants + Organic fertilizer were applied, with cob lengths of 18.95 cm.

The cobs diameter did not show differences in the treatments applied, obtaining the highest values in the complete fertilization treatment (39 mm) and the lowest value was reported by the omission of N (34 mm), these are below the data reported by Guamán et al. (2020), where they report a cob diameter of 45 mm for the hybrid INIAP H-551.

The percentage of rotten cobs did not show differences; however, the highest values were observed with the S omission treatment (56%) and the lowest value with the Mg omission treatment (49%).

Table 2. Average plant height, ear height, days to flowering, cob length, cob diameter, 100-seed weight, and percentage of rotten ears, affected by nutrient omission in breeding a new simple hybrid of durum corn (*Zea mays* L.). Rainy season.

Treatment	Plant height (cm)	Ear height (cm)	Flowering ♂	Flowering ♀	Cob length (cm)	Cob diameter (mm)	Weight 100 seeds (g)	Rotten cob (%)
PKSMgB (-N)	173	85	64	61	15	34	23	38
NKSMgB (-P)	182	112	64	60	15	35	21	38
NPSMgB (-K)	183	101	63	60	16	36	22	37
NPKMgB (-S)	190	96	64	60	15	36	23	56
NPKSB (-Mg)	193	102	63	60	16	35	24	34
NPKSMg (-B)	185	102	63	61	14	36	26	49
NPKSMgB	183	92	62	60	15	39	27	42
PFP*	194	97	63	60	15	36	23	44
Witness	170	85	62	60	14	37	23	40
Average	184	97	63	60	15	36	23	42
Significance	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	5.86	13.31	1.76	2.43	8.53	7.04	11.5	25.94

* PFP= Producer's fertilization plot; NS: Not significant; CV= Coefficient of variation.

The variables stem diameter, stem lodging and root, presented highly significant statistical differences ($P<0.05$) (Table 3). The greatest stem diameter reduction was found with the N omission treatment (19 mm); however, the omission of Mg obtained the greatest diameter (22 mm), these values are similar to those reported by Remache et al. (2017), indicating that with N omission a stem diameter of 17 mm was obtained.

Regarding the stem and root lodging variables, the highest percentages were observed with the N omission treatment at 28.71 and 5.86%, respectively, with the lowest percentages of root lodging observed with the omission of 13.55% (K) and with the absence of 1.68% (B). These results were corroborated by Mateus et al. (2020), who state that high N application rates in no-tillage promote higher nutrient concentrations in leaves (mainly N and P), corn yield and production because it favors stalk development; whereas, with a limitation in this nutrient, it can result in weak stalks and roots.

Effect of Nutrient Omission on Chlorophyll Levels

Table 4 shows the chlorophyll levels at 25, 30 and 35 DAS, highly significant differences were found, registering the lowest values in the omission of N (36.68, 39.55 and 39 atLEAF units), respectively. These findings align with Ontiveros-Capurata et al. (2022), stated, that atLEAF meters can be used to

Table 3. Average stalk diameter, stalk and root lodging affected by nutrient omission in breeding a new simple hybrid of durum maize (*Zea mays* L.). Rainy season.

Treatment	Stem diameter (mm)	Stem stalks (%)	Root lodging (%)
PKSMgB (-N)	19.00^{C}	28.71^{A}	5.86^{A}
NKSMgB (-P)	21.00^{AB}	18.54^{AB}	1.95^{C}
NPSMgB (-K)	22.00^{A}	13.55^{B}	2.51^{BC}
NPKMgB (-S)	22.00^{A}	14.91^{B}	1.72^{C}
NPKSB (-Mg)	22.00^{A}	20.00^{AB}	2.20^{C}
NPKSMg (-B)	20.00^{ABC}	20.62^{AB}	1.68^{C}
NPKSMgB	21.00^{BC}	17.27^{B}	3.66^{BC}
PFP*	21.00^{ABC}	22.92^{AB}	2.17^{C}
Witness	19.00^{BC}	21.69^{AB}	4.49^{AB}
Average	21.00	19.80	2.92
Significance	**	**	**
CV (%)	5.34	22.12	30.52

*PFP= Producer's fertilization plot; CV= Coefficient of variation; Means with the same letter do not differ statistically according to Tukey's test at 1%.

estimate N levels in plants in a non-destructive, fast and reliable way. The observed reduction in chlorophyll content due to N omission is consistent with Toor et al. (2021), who state that N is an important component of chlorophyll, proteins, nucleic acids and other plant components. These findings underscore this importance and highlight the need to consider nitrogen as a key element in plant nutrition.

Table 4. Chlorophyll levels in atLEAF units as affected by nutrient omission in breeding a new simple hybrid of durum maize (*Zea mays* L.). Rainy season.

Treatments	atLEAF units		
	25 DAS	30 DAS	35 DAS
PKSMgB (-N)	36.68^{B}	39.55^{B}	39.00^{B}
NKSMgB (-P)	47.68^{A}	44.54^{AB}	46.81^{A}
NPSMgB (-K)	47.09^{A}	47.06^{A}	46.86^{A}
NPKMgB (-S)	44.85^{A}	47.29^{A}	45.55^{A}
NPKSB (-Mg)	44.04^{A}	47.06^{A}	45.29^{A}
NPKSMg (-B)	46.07^{A}	47.44^{A}	44.70^{A}
NPKSMgB	46.56^{A}	46.59^{A}	46.72^{A}
PFP*: NPK	48.05^{A}	46.39^{A}	45.86^{A}
Witness	45.25^{A}	43.69^{AB}	45.48^{A}
Average	45.14	45.51	45.14
Significance	**	**	**
CV (%)	6.06	5.05	4.97

* PFP= Producer's fertilization plot; CV= Coefficient of variation; Means with the same letter do not differ statistically according to Tukey's test at 1%.

Seed yield due to nutrient omission plot

The yield obtained in this research showed significant statistical differences, due to the effect of nutrient omissions (Figure 1). The treatment with the omission of B (2,093 kg ha^{-1}) was the highest, and the lowest due to the effect of omissions with Mg with 1,126 kg ha^{-1}; these values are lower than those reported by Barrios-Sánchez et al. (2019), since they worked with the commercial hybrid DK-5277, yields of 10,274 kg ha^{-1} were obtained by applying nitrogen fertilization, which is typical for commercial materials. However, this is not the case for genetic materials with a high degree of homozygosity, which tends to produce very little dry matter (Gutiérrez et al 2016). Therefore, these results align with those found by Hernández et al. (2010), who, when evaluating 17 homozygous lines of maize parent lines in Venezuela, obtained yields ranging from 1,707 to 3,199 kg ha^{-1}.

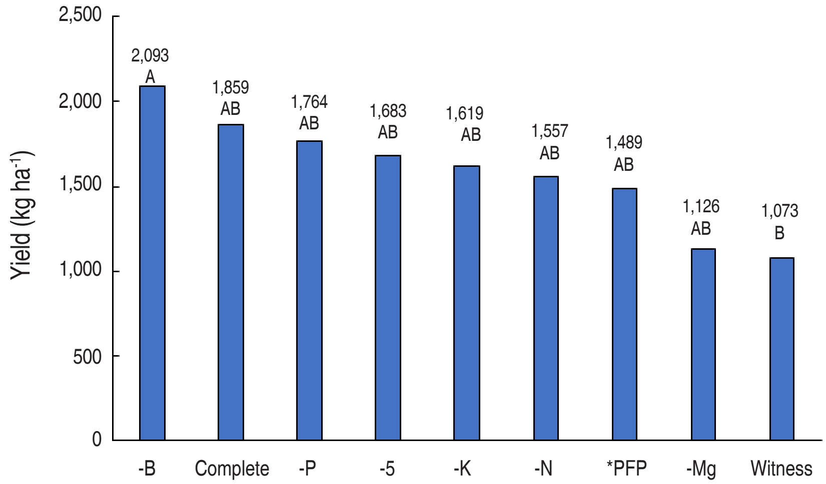

Figure 1. Yield of durum corn (*Zea mays* L.) affected by nutrient omission for breeding a new single hybrid. rainy season; * PFP= Producer's fertilization plot.

Effect of nutrient omission on fertilization efficiencies

The partial productivity factor (PPF) is given by the relationship between the kg of seed produced and the kg of element applied, as described in Table 5. The results indicate that the omission of key nutrients such as N, B and Mg affects the partial productivity factor (PPF). The omission of B generally increased the PPF, while the omission of Mg had the greatest negative impact, especially on the productivity of N and K, this is discussed below.

In the partial factor of N productivity, the highest effect was found with the omission of B (14.95 kg kg^{-1}) and the lowest with the omission of Mg (7.95 kg kg^{-1}), which means that for each kg of B, the yield decreases by 11.17% the PPF of N in relation to the FC treatment. For P, the PPF, with the omission of B was achieved 45.50 kg kg^{-1} P, indicating that the effect of the application of the element, reduces by 11.02% the PPF of P, in relation to the FC treatment. The application of Mg caused an increase in P PPF of 41.03%. The PPF of K was found that, with the omission of B, the highest PPF was achieved (34.89 kg kg^{-1} of K), indicating a reduction of 12.62% of harvested grain per kg of K applied with the addition of B, in relation to the FC. The lowest FPP of K resulted from the omission of Mg (18.27 kg kg^{-1}), being that with its application there is an increase in FPP of K of 41.02%, these results coincide with the reported by Hasang et al. (2018). For S, the PPF, the omission of B with 47.57 kg kg^{-1} of S applied, resulted in the highest PPF value; this implies that with the application of B the PPF of S will be reduced by 42.02%, as a function of FC.

For Mg, it was found that the treatment with omission of B (38.06 kg kg^{-1}), had the highest Mg PPF, showing a 12.63% decrease in Mg PPF due to the application of B, when compared to the complete fertilization treatment. Also, it can be seen that the omission of N affected the Mg PPF, presenting the lowest value (24.75 kg kg^{-1} of Mg).

Therefore, the application of N increased the PPF of Mg by 26.75% in relation to the CF treatment.

It is observed that the PPF of B, which with complete fertilization 1,239 kg kg^{-1} of B was achieved and proved to be the highest; on the other hand, the omission of Mg presented the lowest value of PPF of B with 730 kg kg^{-1}, this indicates that if Mg is applied the PPF of B is increased by 41.08%. These results do not agree with that found by Kumar et al. (2020) who found that the omission of N, P and B nutrients in wheat crops significantly reduced yield, achieving an increase in the dose of nutrients applied based on soil sampling resulted in an increase of about 14-17% in wheat grain yield as compared to farmers.

Table 5. Partial productivity factor (kg kg^{-1}) as affected by nutrient omission in the breeding of a new simple hybrid of durum corn (*Zea mays* L.). Rainy season.

Treatment	N	P	K	S	Mg	B
	(kg of grain harvested / kg of element applied)					
PKSMgZnB (-N)	0.00	29.59	22.69	30.94	24.75	907.00
NKSMgZnB (-P)	10.49	0.00	24.49	33.39	26.71	979.00
NPSMgZnB (-K)	10.40	31.64	0.00	33.08	26.46	970,00
NPKMgZnB (-S)	12.02	36.58	28.05	0.00	30.60	1,121.00
NPKSZnB (-Mg)	7.83	23.83	18.27	24.91	0.00	730.00
NPKSMgZn (-B)	14.95	45.50	34.89	47.57	38.06	0.00
NPKSMgZnB (FC)	13.28	40.40	30.98	42.24	33.79	1,239.00
PFP*	8.04	24.48	18.77	0.00	0.00	0.00
Witness	0.00	0.00	0.00	0.00	0.00	0.00
Average	8.56	25.78	19.79	23.57	20.04	661.01
Maximum	14.95	45.50	34.89	47.57	38,06	1,239.00
Minimum	7.83	23.83	18.27	24.91	24.75	730.00

* PFP= Producer's fertilization plot.

The agronomic efficiency (AE) is given by the ratio between the kg increase in production and the kg of element applied, as described in Table 6. The agronomic efficiency of all the elements analyzed was negatively affected by nutrient omissions, with Mg having the most significant adverse impact, particularly on AE S and AE P. These findings are detailed below.

The highest N AE (3.55 kg grain increases kg^{-1} of N applied) was achieved with the FC treatment, followed by the omission of B with (1.68 kg kg^{-1} of N), being negatively affected to a greater degree with the omission of Mg which presented an N AE of -5.45 kg kg^{-1}, which means a loss of 9 kg kg^{-1} of N AE, corresponding to 253.52%. The results are lower than those reported by Snyder and Bruulsema (2007), who report averages between 10 and 30 units of corn grain per unit of applied N, on the other hand, Zamudio et al. (2015), obtained EA of 20.9 kg of corn grain kg^{-1} of N applied to the soil; considering that homozygous lines were used in this research.

The P as well as N resulting in the FC treatment showed the highest AE value (8.46 kg kg^{-1} of P), followed by the omission of B with 5.10 kg kg^{-1} of P, which is mostly reduced by 25.03 kg kg^{-1} (295.86%) with the omission of Mg which presented AE of P of -16.57 kg kg^{-1}.

The maximum K AE was found in the complete fertilization treatment with a 6.72 kg yield increase per kg K applied, while with the omission of Mg was the lowest with -12.71 kg kg^{-1} of K; reducing by 19.43 kg kg^{-1} of K (289.13%). These results coincide with the values reported by Hasang et al. (2018) with a decrease in EA of 10.39 kg kg^{-1} of K in the Mg omission treatment.

The B omission treatment showed an S AE of 5.33 kg kg^{-1} of S, which was the highest; on the other hand, the lowest AE was obtained with the omission of Mg (-17.33 kg kg^{-1} of S), which indicates that it tends to decrease by 22.66 kg kg^{-1} of S (425.14%), followed by the (-N) treatment with a decrease of 16.66 kg kg^{-1} of S (312.57%).

The highest agronomic efficiency of Mg was found with the FC treatment with 13.86 kg kg^{-1} of Mg; on the other hand, the treatment with the omission of N reduced the EA of S the most, presenting -9.04 kg kg^{-1} of Mg; therefore, it showed a reduction of 22.9 kg for every kilo of S applied, equivalent to 165.22, these results are similar to those found by Hasang et al. (2018) who report that with the omission of N there was a decrease in the EA of Mg of 10.6 kg kg^{-1}.

For B, the efficiencies were negative for all the omitted elements. The lowest decrease was observed with the S omission treatment with values of -117.14 kg kg^{-1} and a reduction in efficiency was observed with the omission of Mg with -508.24 kg kg^{-1}. These values do not coincide with those obtained by Hasang et al. (2018) who reported that with the omission of N there was a reduction of 300 kg kg^{-1} of B.

Table 6. Agronomic efficiency (kg kg^{-1}). affected by nutrient omission in the breeding of a new simple hybrid of durum corn (*Zea mays* L.). Rainy season.

Treatment	N	P	K	S	Mg	B
	(kg increase in production/kg element applied)					
PKSMgZnB (-N)	0.00	-10.81	-8.29	-11.30	-9.04	-331.55
NKSMgZnB (-P)	-2.78	0.00	-6.49	-8.85	-7.08	-259.55
NPSMgZnB (-K)	-2.88	-8.77	0.00	-9.16	-7.33	-268.84
NPKMgZnB (-S)	-1.26	-3.82	-2.93	0.00	-3.19	-117.14
NPKSZnB (-Mg)	-5.45	-16.57	-12.71	-17.33	0.00	-508.24
NPKSMgZn (-B)	1.68	5.10	3.91	5.33	4.27	0.00
NPKSMgZnB (FC)	3.55	8.46	6.72	3.99	13.86	-156.39
PFP*	-5.23	-15.92	-12.21	0.00	0.00	0.00
Witness	0.00	0.00	0.00	0.00	0.00	0.00
Average	0.49	5.64	0.77	4.59	2.49	192.40
Maximum	3.55	8.46	6.72	5.33	13.86	-508.24
Minimum	-5.23	-16.57	-12.71	-17.33	-9.04	-117.14

* PFP= Producer's fertilizer plot.

The nutrient harvest index (NHI) indicates the proportion of nutrients that are absorbed that will be exported to the grain (Table 7). The HI of N in the omission of nitrogen registered the highest HI with 0.36, followed by the omission of B (0.29). For P, the highest HI was observed with the treatment with omission of B with values of 0.44, with the lowest HI being seen with the -S treatment with 0.17. Conversely, the HI of K showed that the treatment with omission of B had the most significant impact on its HI, presenting a value of 0.14. The omission of sulfur negatively affected the HI of Mg (0.10); however, the omission of N, P and B obtained the highest HI of sulfur.

The HI of B obtained the lowest value with the omission of S (0.5); in contrast, the omission of nitrogen obtained the highest average of 0.20. These harvest indices are lower than those found by Zamudio et al. (2016), who, when evaluating the hybrid H-51 under different densities and doses of nitrogen fertilization, reported IHN=0.45, IHP=0.75, IHK=0.21, IHMg=0.45, and IHS=0.43. The omission of nutrients, especially nitrogen and boron, impacts the translocation of essential elements to the grain. The greater capacity of nitrogen to be transported under deficiency conditions suggests a prioritization of this nutrient to optimize grain production, while the negative influence of sulfur omission on the magnesium

Table 7. Nutrient harvest index affected by the omission of nutrients in the breeding of a new simple hybrid of durum corn (*Zea mays* L.). Rainy season.

Treatment	N	P	K	Mg	S	B
	Harvest index					
PKSMgZnB (-N)	0.36	0.37	0.09	0.24	0.14	0.20
NKSMgZnB (-P)	0.22	0.32	0.06	0.18	0.14	0.10
NPSMgZnB (-K)	0.21	0.34	0.09	0.17	0.10	0.08
NPKMgZnB (-S)	0.10	0.17	0.05	0.10	0.05	0.05
NPKSZnB (-Mg)	0.16	0.27	0.06	0.12	0.07	0.11
NPKSMgZn (-B)	0.29	0.44	0.14	0.26	0.14	0.11
NPKSMgZnB (FC)	0.14	0.33	0.06	0.15	0.07	0.13
PFP*	0.22	0.30	0.06	0.17	0.08	0.13
Witness	0.23	0.33	0.13	0.22	0.13	0.09
Average	0.21	0.32	0.08	0.18	0.10	0.11
Maximum	0.36	0.44	0.14	0.26	0.14	0.20
Minimum	0.10	0.17	0.05	0.10	0.05	0.05

* PFP= Producer's fertilization plot.

harvest index highlights the interdependence of these nutrients.

CONCLUSION

The potential seed yield of the newly developed simple corn hybrid was 2,093 kg ha^{-1}, which is understandable given the high homozygosity of the genetic materials. However, this yield was affected by the omission of Mg, resulting in a 46.20% reduction, making it the most limiting nutrient for seed production in the Mocache area. Additionally, nitrogen (N) was the element that most impacted the agronomic traits of corn. On the other hand, the omission of boron (B) resulted in the highest seed yield, as its inclusion in the fertilization plan led to a reduction in the PPF and AE of the studied elements. In these soils, the natural levels of boron are sufficient, and its excess negatively affects the efficiencies. The fertilization priorities for the soils in Mocache follow the sequence Mg>N>K=P>S>>B. These results highlight the importance of proper Mg and N management in fertilization programs to maximize corn seed yield, suggesting that future research should focus on the interaction of these nutrients under different agroecological conditions and their impact on agronomic efficiency. Thus, optimizing corn fertilization is crucial, not only to enhance production but also to reduce costs and mitigate contamination risks. Given that corn is a strategic crop for food security, efficient nutrient management will play a vital role in ensuring sustainable agricultural practices and maximizing yields while protecting the environment.

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Research article

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Effect of foliar stimulant biofortified with bamboo in tomato cultivation yield (*Solanum lycopersicum* L.)

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto del estimulante foliar biofortificado con bambú en el rendimiento del cultivo de tomate (*Solanum lycopersicum* L.)

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ABSTRACT

Keywords:
Bamboo
Biostimulant
Hybrids
Tomato yield

Biostimulants promote the physiological balance of plants, favoring the expression of genetic potential to improve the production and quality of crops. This research aimed to evaluate the morphological and yield behavior with the application of a foliar stimulant biofortified with bamboo in two tomato hybrids, under semi-protected conditions. The research was carried out in the Las Piedras facility, Pedro Carbo canton, Guayas province, Ecuador. A completely randomized block experimental design with a 2x3 factorial arrangement with 4 repetitions was used. The treatments were: control, biostimulant and biostimulant + mineral fertilization (N). Morphological behavior and performance variables (t ha^{-1}) were evaluated. The results obtained showed that with biostimulant treatment + mineral fertilization (N), the tomato plants with "Super Kalel" hybrid responded favorably in terms of morphological characteristics, with an average yield of 74 t ha^{-1}. Therefore, the biofortified stimulant with bamboo improves the physiological processes of plants, which leads to good harvest yields.

RESUMEN

Palabras clave:
Bambú
Bioestimulante
Híbridos
Rendimiento de tomate

Los bioestimulantes promueven el equilibrio fisiológico de las plantas, favoreciendo la expresión del potencial genético para mejorar la producción y calidad de las cosechas. El objetivo de esta investigación fue evaluar el comportamiento morfológico y de rendimiento con la aplicación de un estimulante foliar biofortificado con bambú en dos híbridos de tomate, bajo condiciones semiprotegidas. La investigación se realizó en el recinto Las Piedras, cantón Pedro Carbo, provincia del Guayas, Ecuador. Se utilizó un diseño experimental de bloques completamente aleatorio con arreglo factorial de 2x3 con 4 repeticiones. Los tratamientos fueron: testigo, bioestimulante y bioestimulante + fertilización mineral (N). Se evaluaron variables de comportamiento morfológico y rendimiento (t ha^{-1}). Los resultados obtenidos mostraron que el tratamiento de bioestimulante + fertilización mineral (N), las plantas de tomate con el híbrido "Super Kalel" respondieron favorablemente en las características morfológicas, con un rendimiento promedio de 74 t ha^{-1}. Por lo tanto, el estimulante biofortificado con bambú mejora los procesos fisiológicos de las plantas lo que conlleva a obtener buenos rendimientos de cosecha.

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The tomato (*Solanum lycopersicum* L.) is the most widely grown horticultural product in the world; its consumption has benefits to human health due to the antioxidant content (Estrada-Arellano et al. 2022), is an essential source of certain minerals content in vitamins such as B1, B2, B5 and vitamin C, has carotenoids and lycopene, these components are antioxidants that act as a protective function of human cells (Blanco 2019).

Foliar fertilization is an agricultural practice that serves to correct nutritional deficiencies in plants that are under stress conditions or in soils with low availability of nutrients. It also consists of applying nutrient solutions directly to the leaves (Murillo et al. 2013).

To ensure the nutritional quality of food, agronomic biofortification strategies must be implemented (Cedeño et al. 2018). Organic agricultural practices (provision of organic biostimulants to the soil or leaf area) are tools that reduce stress, provide resistance to pests and diseases, and increase metabolic and photosynthetic rates (Mendoza et al. 2014).

Biostimulants are applied to plants by foliar or edaphic route, stimulating natural processes that increase nutrient uptake (Dehkordi et al. 2021). They also offer solutions to improve fertilization plans and food security; these support the capacity of biological systems for nutrient scarcity problems (Lallié et al. 2021). On the other hand, López (2008) mentions that species such as *Guadua angustifolia* Kunth, contribute to reducing soil erosion. It protects the soil by having more than 20 kg^{-1} of roots that stop the vegetation layer, promotes water regulation by storing up to 30,000 L ha^{-1} of water, and increases the content of organic matter by providing about 30 t ha^{-1} of biomass. Bamboo biomass has favorable nutritional characteristics to be used in soil recovery. It contributes to increased biological activity and soil structure (Cairo et al. 2018).

According to studies conducted by Alvarez et al. (2014) and Cairo-Cairo et al. (2017), where they characterized different bamboo substrates indicated that elements such as C, N, P, K, Ca, and Mg are found to demonstrate outstanding qualities to be used in the improvement of erosinous soils; however, there is no biofertilizer biofortified with bamboo that is used for the stimulation of growth, development and yield in crops.

Based on the above, this research study aims to obtain results that improve the productivity of tomato crops through the application of a stimulant biofortified with bamboo, in order to improve yield and fruit quality.

MATERIALS AND METHODS

Study Location

This research was carried out in the "Las Piedras" precinct, Sabanilla parish, Pedro Carbo canton, Guayas province, Ecuador, from April to August 2022. Located at an altitude of 56 meters above sea level (masl) and located at coordinates 1°50′00"S 80°14′00"W. The climatological parameters are shown in (Table 1), and the results were obtained through the POWER Data Access Viewer.

Table 1. Climatological parameters of the experimental site during the study.

Months	Temperature (°C)			Shortwave radiation index - All sky	Shortwave radiation index- Clear sky	Total active photosynthetic radiation - Entire sky	Wind speed (m s^{-1})			Relative humidity	Precipitation
	Min.	Max.	Stocking	MJ/M2/DIA (MJ)	MJ/M2/DIA	MJ/M2/DIA	Min.	Max.	Stocking	(%)	(mm)
April	21.66	31.38	26.52	2.10	131.61	91.97	1.02	3.05	2.04	80.53	2.55
May	21.54	34.39	27.97	1.47	124.09	62.68	1.24	3.64	2.44	69.78	0.72
June	20.98	34.83	27.91	1.21	118.34	54.81	1.40	3.92	2.66	62.44	0.38
July	20.58	35.97	28.27	1.22	119.49	57.46	1.70	4.61	3.16	59.40	0.33
August	20.72	36.85	28.78	1.35	124.80	63.05	1.72	4.94	3.33	57.53	0.10

Soil sample collection and analysis from the experimental site

To collect soil samples, it was applied the methodology posed by Mendoza and Espinoza (2017). As for the chemical characterization of the soil, various analytical procedures adjusted to the particularities of each element and parameter were used. Regarding the determination of NH_4 and P, it was done by colorimetry with the modified Olsen extractant with a pH of 8.5. The cations K, Ca, Mg, Zn, Cu, Fe and Mn were quantified by atomic absorption with modified Olsen at pH=8.5 as well, which allows a determination of exchangeable cations. The S content was determined by turbidimetry with monobasic calcium phosphate as extractant, while B was determined by colorimetry using the same extractant. The Cl concentration was quantified by volumetry using a saturated soil dough with water in a ratio of 1:2.5. Finally, soil pH is also required by potentiometry in a saturated soil dough (1:2.5), as a standard method for evaluating soil acidity or alkalinity. The results of the physicochemical characteristics of the experimental site are detailed in (Table 2).

Table 2. The result of the soil analysis of the experimental unit.

Terra in data		NH_4	P	K	AC	Mg	S	Zn	Cu	Faith	Mn	B
		(µg mL)										
Place	Pedro Carbo-Pebbles	25M	107A	858A	5,285A	365A	37A	5.3M	9.9A	18B	29A	0.90M

M.O	pH	Ca/Mg	mg K^{-1}	Ca+Mg/K	∑ Bases	Texture (%)			Texture Class
%		MEQ 100 mL				sand	slime	clay	
4.13M	7.3NP	8.80 A	1.37 B	13.38 M	31.63	42	34	24	Frank

PN: Prac. Neutral; M.O: Organic matter, A: High, M: Medium, B: Low.

Elaboration of the biostimulant biofortified with bamboo used in the research

The biostimulant was prepared with tender bamboo canes and transverse cuts of 15 cm long x 5 cm wide were made from each of the canes until a mass of 1 kg^{-1} was obtained; then it was deposited in a 20 L bucket, along with solid mountain microorganisms (0.45 kg^{-1}) plus liquid mountain microorganisms (1 L), taking as reference what was reported by Castro-Barquero and González-Acuña (2021), it was added molasses, in a volume of 2 L, finally a surface water source (15 L) was taken from the Pedro Carbo river in December because it is winter and the flows of surface water sources increase, which causes a natural decrease phenomenon of water salts and then it was allowed to rest for 22 days, the entire complex was filtered until obtaining the biofortified stimulant (20 L), then the nutritional content was analyzed, results that were analyzed at the INIAP-Costa. South-Ecuador Laboratory and its composition are detailed in Table 3.

Table 3. Result of the nutritional analysis of the bamboo-based biostimulant.

Sample Identification	N	P	K	AC	Mg	Cu	Faith	Mn	Zn
		ppm							
Biostimulant	23	544	10,253	2,591	1,154	3	143	17	8

Extractant and methodology: Total N (%): Mixture with sulphuric acid (Microkjeldah); P: Wet digestion (Colorimetry); K-Ca-Mg-Cu-Fe-Mn-Zn: Wet Digestion (Atomic Absorption).

Characteristics of tomato hybrids used in the research

Two F1 hybrids, Kalel and La Roca, were used, and their characteristics are as follows: F1 Kalel is an indeterminate hybrid of high yield; it has round red fruits with a mass of 250-300 g; its growth is indeterminate with short internodes and dark green leaf; it is tolerant to TYLCV, TMV, Fol, and Vd (Agrinova 2019). La Roca is a hybrid with excellent coverage; it has fruit with an average mass of 130 g and is resistant to V ToMV, Fol, N, TYLCV, and TSWV (NIRIT SEEDS LTD 2019).

Treatment design

A completely randomized block design was used with a factorial arrangement of 2 (hybrids) x 3 ("nutritional components" and nitrogen was added to the element because according to the soil analysis, it did not satisfy the nutritional coefficient of tomato cultivation), with four repetitions giving a total of 24 experimental units, as shown in (Table 4).

Research Management: before preparing the land, a plastic tunnel with dimensions of 6 m wide x 30 m long was made, placing a milky white agricultural plastic in the aeri part with 50% of protection against ultraviolet radiation.

Table 4. Treatments Evaluated in Research.

Treatments	Hybrids	Dose Biost - N	Combination factorial
1	Super Kalel	0	H1-C1
2	Super Kalel	2 L ha^{-1}	H1- C2
3	Super Kalel	2 L ha^{-1} + 392 kg ha^{-1} N	H1-C3
4	The Rock	0	H2-C1
5	The Rock	2 L ha^{-1}	H2-C2
6	The Rock	2 L ha^{-1} + 392 kg ha^{-1} N	H2-C3

H: Hybrid; C: Nutritional component; Biost: Biostimulant; N: Nitrogen. Treatments were considered according to soil and biostimulant analysis reports.

Soil preparation: It was done by plowing, then a targeted soil amendment was applied using 20 kg ha^{-1} of ferrous sulfate to meet the need for Fe (Table 2); in addition, 10 L ha^{-1} of humic acid was applied to condition the soil and allow better absorption of elements for the crop. This procedure was performed 30 days before the transplant.

Sowing: it was carried out by transplanting with seedlings that were 21 days old, and a sowing distance of 0.6 m between plants and 1.4 m between rows was considered, giving a total of 11,905 plants ha^{-1}.

Irrigation: a drip irrigation system was implemented whose emitter had a flow rate of 3 L h^{-1}, applying 2 h of irrigation daily, twice a week for a total of twelve weeks, which means 12 L/week/plant, that is, 144 L/plant/production cycle, equivalent to an irrigation sheet of 171.4 mm with the planting mentioned above distance.

Fertilization: it was carried out according to the amount of nutrient absorbed and extracted expressed in kg of nutrient per ton of organ harvested for tomato cultivation, taking as a reference what was indicated by Ciampitti and García (2007), together with the interpretation of the soil analysis, the nitrogen was at an average level (Table 2). 392 kg ha^{-1} of N was chosen to meet the needs; it was used as a source of nitrogen fertilizer (Urea: 733 kg ha^{-1}), with two fractional applications of N (25 days after sowing) and (40 days after sowing). Foliar application was carried out using the biostimulant with two applications, one at 28 days (1 L ha^{-1}) and the second at 48 days (1 L ha^{-1}).

Phytosanitary management: it was carried out according to the presence of pests and diseases therefore, 30 days after transplantation, it was found (*Helicoverpa armigera*), and for its control, Spinetoram was applied in doses of 100 cc ha^{-1}, and at 74 days after transplantation it was found (powdery mildew*)* Mancozeb was applied in doses 1.5 kg ha^{-1}. Weed control was manual with the use of a machete.

Tutoring: it was done 30 days after transplanting, and with an ice axe, it was tied to the lower part of the stem, wrapping around the plant, and then connected with the tempered wire along the row.

Pruning: two types of pruning were carried out; the first was the pruning of suckers of the axillary meristematic buds 5 cm in length; it was carried out twice a week from the vegetative to the reproductive state. The second pruning was defoliation (old or damaged leaves).

Harvest: it was carried out when the fruits presented the characteristic symptoms of physiological maturity, which occurred at 107 days for the two hybrids.

Variables evaluated

The agronomic variables evaluated during the research are reflected in the (Table 5).

Table 5. Description of the variables of tomato cultivation.

Variables	Description
Floor height (m)	It was measured with the help of a tape measure and proceeded to measure from the neck of the root to the apical bud of the plant.
Stem diameter (cm)	It was done with the help of a Vernier caliper, 5 cm high from the base of the stem.
Days to harvest	This variable was taken when the fruits presented the characteristic symptoms of physiological maturity; that is, when the fruits presented the 4:30 degree, at 60% of the tomato surface presented a red color.
Average Fruit Mass (g):	The fruits were weighed using a digital scale, expressed in grams, and were evaluated at the time of harvest.
Polar fruit diameter (cm)	It was taken with a vernier caliper; this variable was taken at ten fruits per plot.
Equatorial fruit diameter (cm)	The diameter of the equatorial fruit was measured in centimeters, with a vernier caliper; this variable was taken at ten fruits per plot.
Number of fruits per plant	For this variable, five plants were chosen for each plot. Moreover, the fruit count was carried out.
Yield (t ha^{-1})	The yields in kilograms of each experimental area were expressed in a net area of 3.9 m^2 and then transformed to t ha^{-1} the following mathematical formula. R= (PFP*10,000 m^2)/ANC Where: R = yield (t ha^{-1}), MFP= fruit mass per plot (kg^{-1}), ANC = net harvested area.

Data Analysis

To obtain the data for each of the treatments based on the variables, a random sampling of ten plants was applied for the agronomic variables, and for the yield, it was selected the net harvested area. After that, the evaluated variables were subjected to the analysis of variance, while the statistically significant differences between the means of each treatment was determined through the Tukey probability test (P<0.05) using the InfoStat statistical software in its 2020 version.

RESULTS AND DISCUSSION

Evaluation of growth variables

In plant height, it was observed that the Super Kalel hybrid obtained the highest plant height with an average of 2.71 m, while the La Roca hybrid reached a height of 2.39 m, thus giving hybrids and nutritional components that presented high statistical significance in the interaction of hybrid and nutrients they did not present statistical significance, with a coefficient of variation of 4.38% (Table 6). These results are superior to those reported by Mendoza et al. (2023) who evaluated tomato hybrids such as “Miramar”; and “Pietro” under protected conditions and obtained values of: 1.65 m, 1.56 m with a synthetic fertilization.

In the treatment with the application of Biost + N, tomato plants managed to obtain a higher plant height with an average of 2.74 m, a higher value than the work carried out by Agudelo and Polanco (2019), who evaluated a foliar biostimulant in the production of “Chonto” type tomato in two growing environments and obtained an average of 1.15 m, as well as the research conducted by Castillo-Ferrer et al. (2022) who evaluated different fertilizers and using efficient microorganisms (EM) in doses 10 L ha^{-1} obtained a value of 0.97 m with the tomato variety “Celeste F-2 from Skay Way”.

Regarding the variable of stem diameter, it can be observed that there is no statistical difference between hybrids and the interaction of hybrids plus nutritional component, while there is high statistical significance in the nutritional component effect, with a coefficient of variation of 7.60% (Table 6).

According to the nutrition factor, with the application of Biost + N, a result of 2.19 cm was obtained, an average higher than the research work carried out by Reyes-Pérez et al. (2022) who evaluated the agrobiological effectiveness of Chitosan in doses of 3 g L^{-1} in the tomato variety "Vyta" obtained a value of 1.13 cm, another research conducted by Villegas-Espinoza et al. (2018) where evaluated a plant biostimulant in concentration of 1/30(v/v) and obtained an average of 1.30 cm.

Tomato harvest days

According to this variable, it was possible to determine that for hybrids plus the interaction of hybrids by nutritional component, there is no statistical difference, while in the nutritional component effect, there is statistical significance, with a coefficient of variation of 0.73% (Table 6).

Table 6. Analysis of the variance effect of the hybrid factor (A) and nutritional components (B) on tomato cultivation.

Treatments	Floor height (m)	Stem diameter (cm)	Days to harvest
Hybrids			
Super Kalel	2.71^{b}	1.90	106
The Rock	2.39^{a}	1.85	108
Nutritional Component			
T	2.36^{a}	1.68^{a}	109^{b}
Biost	2.55^{b}	1.74^{a}	107^{a}
Biost + N	2.74^{c}	2.19^{b}	107^{a}
Interaction of hybrids x nutritional components			
Super Kalel			
T	2.55	1.73	108
Boost	2.71	1.78	107
Biost + N	2.87	2.19	107
The Rock			
T	2.16	1.64	108
Boost	2.39	1.71	108
Biost + N	2.61	2.20	107
C.V	4.38%	7.60%	0.73%
ADEVA *P*-value			
Hybrid Factor (H)	<0.0001 **	0.4025^{NS}	0.5442^{NS}
Nutrition factor (N)	<0.0001 **	<0.0001**	0.031*
Interaction (H) x (N)	0.5705^{NS}	0.7896^{NS}	0.9016^{NS}

NS Not significant at 5% probability of error.
* Significant at 5% chance of error.
** Highly significant at 5% chance of error.
Different letters are statistically different according to Tukey's 5% probability test.

With the use of Biost and Biost + N, the same average was obtained with a value of 107 days to harvest; this result is lower than that reported by Mendoza et al. (2023), who obtained an average of 73 days to harvest with the "Margo" tomato variety after using synthetic fertilization under protected crop conditions.

Performance Parameters

According to this variable of average fruit mass, it can be observed that there is no statistical difference between hybrids and the interaction of hybrids with nutritional components. At the same time, there is a significant difference in the nutritional component effect, with a coefficient of variation of 3.52% (Table 7). With the effect of nutritional components, an average of 207.5 g was obtained with the application of Biost + N, a value higher than that reported by Gabriel-Ortega et al. (2022), who applied biocompost + humus and mycorrhizae in the tomato hybrid "Itaipu" and obtained an average of 76.61 g.

For the variable number of fruits/plants, it was observed that the Super Kalel hybrid obtained the highest number of fruits per plant, with an average of 23, while La Roca had an average of 22. The average fruits by plants are presented in (Table 7), finding in the analysis of variance that, between hybrids and nutritional components, they presented statistical significance. In contrast, the interaction of hybrids and nutritional components did not present statistical significance, with a coefficient of variation of 4.35%. These results are higher than those reported by Reyes-Pérez et al. (2022), who evaluated the agrobiological effectiveness of humic acids at doses of 1:30 v/v in the tomato variety "Floradade" and obtained a value of 6 fruits/plants.

Table 7. Analysis of variance of the hybrid factor and the nutritional component in tomato cultivation.

Treatments	Average fruit mass (g)	Number of fruits/plants
Hybrids		
Super Kalel	169.48	23[b]
The Rock	166.45	22[a]
Nutritional Component		
T	116.32[a]	17[a]
Boost	180.08[b]	23[b]
Biost + N	207.50[c]	27[c]
Interaction of hybrids x nutritional components		
Super Kalel		
T	116.59	18
Boost	183.57	23
Biost + N	208.27	27
The Rock		
T	116.05	17
Biost	176.59	22
Biost + N	206.73	26
C.V	3.52%	4.35%
ADEVA *P*-value		
Hybrid Factor (H)	0.2296[NS]	0.0350*
Nutrition factor (N)	<0.0001**	<0.0001**
Interaction (H) x (N)	0.5189[NS]	0.8048[NS]

[NS] Not significant at 5% probability of error.
* Significant at 5% chance of error.
** Highly significant at 5% chance of error.
Different letters are statistically different according to Tukey's 5% probability test.

With the application of Biost + N, tomato plants obtained a higher number of fruits per plant, with an average value of 27 higher than the research by Gitau et al. (2022) who evaluated the effect of biostimulants based on two Chlorophyta microalgae in the tomato variety "Vilma" and obtained an average of 12 fruits/plants.

In the diameter of the polar fruit, it was possible to determine statistical significance for hybrids, nutritional components, and interaction of hybrids, with a coefficient of variation of 1.09% (Figures 1A-B).

It can be observed that both hybrids obtained a similar average of 5.59 cm for Super Kalel and 5.67 cm for La Roca, a study conducted by Villegas-Espinoza et al. (2018) where evaluated a plant biostimulant in concentration of 1/10(v/v) in the open field in the tomato variety "Amalia" and obtained an average of 5.25 cm, a value that approximates in the hybrids studied.

With the use of Biost + N, an average diameter of 6.33 cm of polar fruit was obtained; this result is more significant than that reported by Jiménez et al. (2009), who evaluated three biostimulant substances for growth and development in the cultivation of tomatoes of the "Vyta" variety and with the use of Chitosan in doses of 150 mg ha^{-1} and obtained an average of 4.72 cm.

According to the interaction of hybrids by nutritional components, the Super Kalel hybrid was determined with the application of Biost + N; an average greater than 6.41 cm was obtained, a value higher than that reported by Reyes-Pérez et al. (2022) when applying humic acids in doses of 1:30 v/v using the tomato variety "Floradade" obtained a value of 5 cm.

For the equatorial fruit diameter variable, it was observed that the Super Kalel hybrid obtained the highest average with 7.10 cm, while La Roca had an average of 6.91 cm, according to the values obtained for the diameter of the equatorial fruit in the (Figures 1C-D). Whether there was statistical significance between hybrids, nutritional components, and interaction with a coefficient of variation of 1.03%. These results are superior to those reported by Rivas-García et al. (2021), who evaluated the application of a QuitoMax® biostimulant in seeds and at the beginning of flowering at a concentration of 1 g L^{-1} in tomato variety L-43 and obtained an average of 4.82 cm.

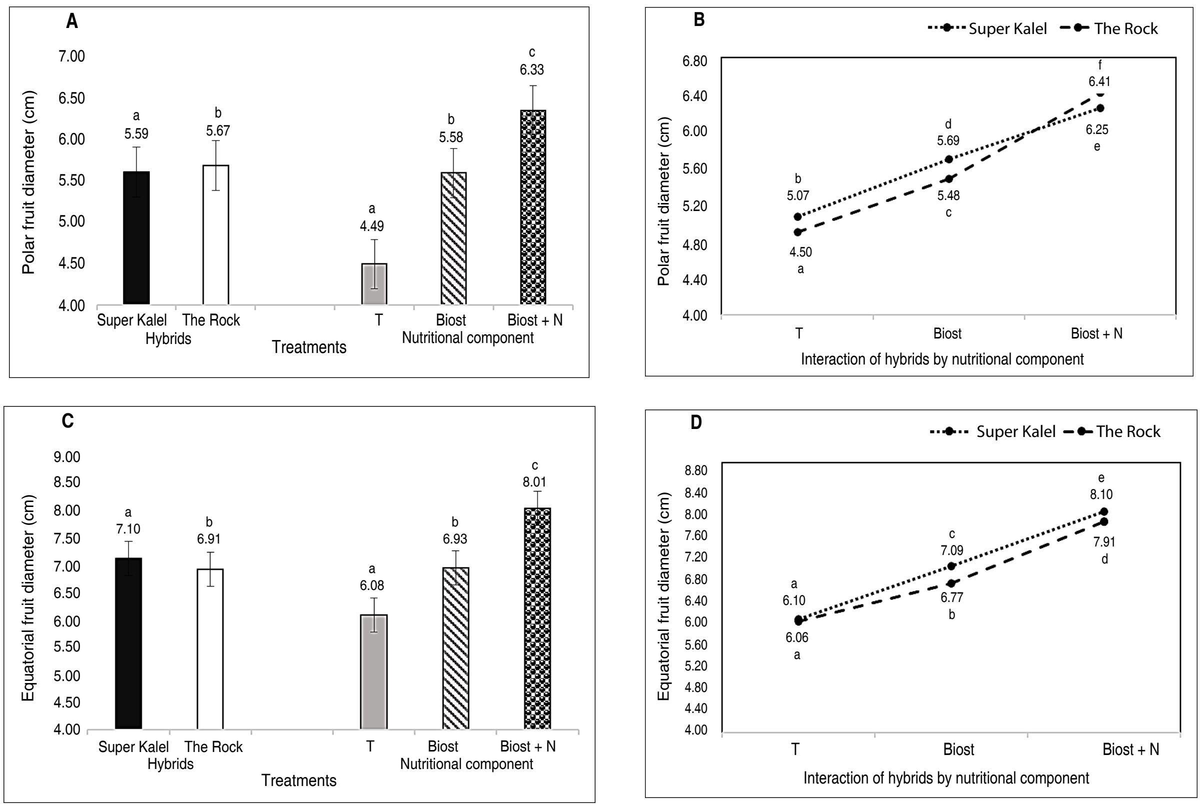

Figure 1. Analysis of variance of the hybrid, nutrition, and interaction factor by the effect of the application of the bamboo-based foliar biostimulant for the variable of polar fruit diameter (cm) (A); (B) and equatorial fruit diameter (cm) (C); (D). Different letters are statistically different according to Tukey's 5% probability test.

With the application of Biost + N, a higher average value of 8.01 cm was obtained, higher than the work carried out by Márquez-Hernández et al. (2013), who applied organic sources of fertilization in greenhouses and with the use of compost plus microelements in irrigation in two tomato genotypes "Bosky" and "Big Beef" and obtained an average of 7.49 cm.

According to the interaction of hybrids by nutritional components, the Super Kalel hybrid was determined with the application of Biost + N and a value greater than 8.10 cm was obtained, an average higher than the work carried out by Boudet et al. (2017) who applied a Bocashi organic fertilizer in the variety "Vyta" in doses of 2.99 t ha^{-1} and obtained an average of 5.53 cm.

For the performance variable (t ha^{-1}), it was possible to determine statistical significance for hybrids, nutritional components, and interaction of hybrids by nutritional component, with a coefficient of variation of 1.03% (Figures 2E-F). It can be observed that the Super Kalel hybrid obtained the highest average with 51.77 t ha^{-1}, while the La Roca hybrid had the lowest average with 49.66 t ha^{-1}; these values are higher than those the study carried out by Campo-Costa et al. (2015) who evaluated the effect of the biostimulant Fitomas-E on plant development and yields of the hybrid tomato crop HA-3019 and obtained a yield of 45 t ha^{-1} with the application of 0.7 L ha^{-1} in seedbed one day before transplanting.

With the effect of nutritional components, it was possible to determine that with the application of Biost + N, it generated a value of 73.15 t ha^{-1}, a higher value in terms of the control and the Biost; this result is higher than that reported by Mendoza et al. (2023) evaluated tomato hybrids such as "Pietro"; "Margo" under conditions protected with synthetic fertilization obtained values of:11.22 and 21.97 t ha^{-1}, respectively.

According to the interaction of hybrids by nutritional components, the Super Kalel hybrid was determined with the application of Biost + N; the highest average was obtained with 74.89 t ha^{-1}, a value higher than the research carried out by Pérez et al. (2017) who evaluated two indeterminate tomato hybrids of the Saladette type "Cid" and "Azhura" and with the application of foliar fertilizers Nutri Humus obtained an average yield of 50 t ha^{-1}.

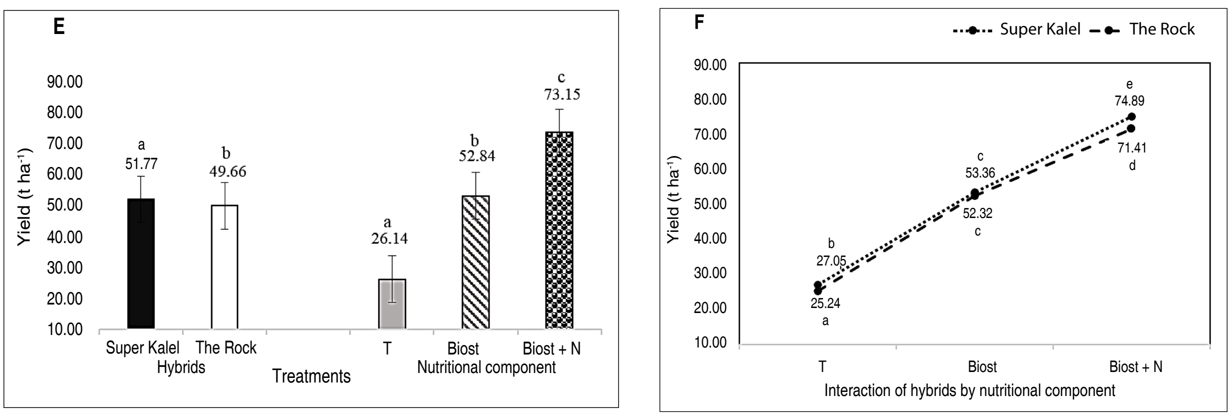

Figure 2. Analysis of variance of the hybrid, nutrition (E), and interaction (F) factor by the effect of the application of the bamboo-based foliar biostimulant for the yield variable (t ha^{-1}). Different letters are statistically different according to Tukey's 5% probability test.

CONCLUSION

The use of the biofortified stimulant with bamboo, together with the addition of nitrogen, generated a favorable response in the morphological variables and yield of the tomato hybrid "Super Kalel" under semi-protected conditions. However, it is necessary to evaluate other tomato hybrids with higher doses of the biofortified stimulant with bamboo applied in this research, to improve tomato harvest yield.

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Revista Facultad Nacional de Agronomía

Effect of intercropping of cabbage, lettuce, parsley and chard on weed incidence and cabbage yield

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto de la asociación repollo, lechuga, perejil y acelga sobre la incidencia de malezas y el rendimiento del repollo

https://doi.org/10.15446/rfnam.v78n1.113113

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ABSTRACT

Keywords:
Brassica oleracea L
Equatorial diameter
Lactuca sativa L
Polar diameter

The present study aimed to evaluate the effect of the association of cabbage, lettuce, parsley and chard on the incidence of weeds and cabbage yield parameters. The research was conducted on the Facultad de Ciencias Agrarias campus of the Universidad de Concepción, Paraguay. The treatments consisted of cabbage (T1), cabbage+lettuce (T2), cabbage+chard (T3), and cabbage+parsley (T4). The trial was laid out in Randomized Complete Block Design (RCBD) with four treatments and six replications, totaling 24 Experimental Units (EUs). Measurements included the percentage of weed incidence, cabbage yield (ton ha^{-1}), and equatorial and polar diameter of the head (cm). These mean values were subjected to analysis of variance (ANOVA) and when significant differences were found between treatments, the mean comparison was performed using the Tukey test at 5%. No significant differences were recorded for cabbage yield, equatorial, and polar diameter (P>0.05). It is concluded that the plant species used in this research in consortium with cabbage positively influenced reducing weed incidence and increasing cabbage yield, but did not significantly affect the equatorial and polar diameters of cabbage heads.

RESUMEN

Palabras clave:
Brassica oleracea L
Diámetro ecuatorial
Lactuca sativa L
Diámetro polar

El presente trabajo tuvo como objetivo evaluar el efecto de la asociación de repollo, lechuga, perejil y acelga sobre la ocurrencia de malezas y parámetros de rendimiento del repollo. La investigación se realizó en el campus de la Facultad de Ciencias Agrarias de la Universidad de Concepción, Paraguay. Los tratamientos utilizados fueron repollo (T1), repollo+lechuga (T2), repollo+acelga (T3) y repollo+perejil (T4). El diseño utilizado fue el de parcelas subdivididas con cuatro tratamientos y seis repeticiones, totalizando 24 Unidades Experimentales (UE). Las determinaciones evaluadas fueron porcentaje de incidencia de malezas, rendimiento del repollo (ton ha^{-1}) y diámetro ecuatorial y polar de la cabeza (cm). Estos valores fueron sometidos a un análisis de la varianza (ANOVA) y, cuando se encontraron diferencias significativas entre los tratamientos, la comparación de medias se realizó mediante la prueba de Tukey al 5%. No se registraron diferencias significativas para el rendimiento del repollo, el diámetro ecuatorial y polar (P>0,05). Se concluye que las especies vegetales utilizadas en este estudio en consorcio con el repollo influyeron positivamente en la reducción de la incidencia de la maleza y en el aumento del rendimiento del repollo, pero no afectaron significativamente a los diámetros ecuatorial y polar del repollo.

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Horticulture is a significant development and economic activity engine with substantial social and economic value. It is a source of employment throughout the entire value chain, plays an important role in regional economies, and is a crucial factor for countries seeking to optimize vegetable consumption in quantity, diversity, and quality (Castagnino et al. 2020).

Like other crops, horticultural species are susceptible to weed interference's negative effects (Fukushi 2016). The weeds most frequently reported by De Egea et al. (2018) in Concepción, Paraguay are *Sagittaria montevidensis, Commelina diffusa* Burm., *Commelina platyphylla* Klotzsch ex Seub., *Cyperus rotundus* L., *Cenchrus equinatus* L., *Echinochloa crus-galli* (L.) O. Schulz, *Digitaria ciliaris* (Retz.) Koern., *Hymenachne amplexicaulis* (Rudge) Nees, *Megathyrsus maximus* (Jacq.), *Amaranthus viridis* L., etc.

The rapid vegetative growth of these weeds has the potential to directly affect crop yield and quality if not controlled in a timely and effective manner (Rojas et al. 2017). Therefore, management practices should be efficient and consider the most appropriate management for the crop (Fukushi 2016).

According to MacLaren et al. (2020) to achieve the goal of sustainable weed management it is suggested that an alternative ecological approach be adopted where the goal is not the total elimination of weeds, but an understanding of what characteristics of agroecosystems confer flexibility to noxious weed invasions and at the same time encourage a diverse weed population to sustain ecosystem services.

In the consortium, the presence of one or more support crops can help suppress weeds by following them without interfering with the development of the crop of economic interest (Castagnino et al. 2020). Therefore, the use of intercropping as a weed management strategy will help reduce the occurrence of weeds and minimize contamination caused by chemical crop protection (Ribas et al. 2020).

Therefore, this research aimed to evaluate the effect of the association and monoculture of cabbage, lettuce, parsley and chard on the occurrence of weeds and cabbage yield parameters.

MATERIALS AND METHODS

The research was conducted on the Facultad de Ciencias Agrarias campus of the Universidad Nacional de Concepción, located 2 km from the city of Concepción, Paraguay. The study was carried out from November 2022 to April 2023. The mean annual precipitation and temperatures can be appreciated in Figure 1.

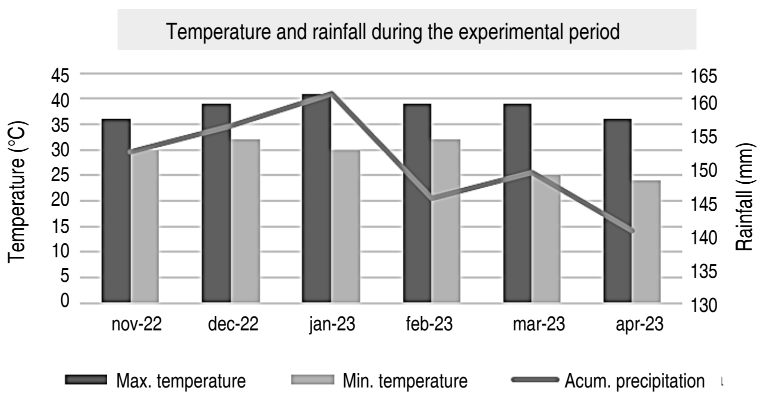

Figure 1. Monthly precipitation and maximum and minimum temperatures during the experimental period (DINAC 2023).

The soil preparation phase started in November. This involved the conventional preparation of the soil, the lifting of the planks, the implementation of a base fertilization regimen, the installation of a drip irrigation system, and the deployment of a half-shade net.

The plant species were planted as follows: the cabbage seeds were initially sown in January. Once the plants had emerged and reached three weeks, two additional species were introduced: parsley and chard. Subsequently, lettuce seedlings that had been cultivated in previously prepared planting trays were transplanted after three weeks. In addition, irrigation was carried out twice a day, once in the morning and once in the afternoon.

The trial was laid out in a Randomized Complete Block Design (RCBD) with four treatments and six replications, totaling 24 Experimental Units (EUs). The dimension of each plot was 3.6 m^2, totaling 155.15 m^2. The treatments (T) consisted of using different vegetable species, with cabbage as the main crop, T1 cabbage (monoculture), T2 (cabbage + lettuce), T3 (cabbage + chard), and T4 (cabbage + parsley).

The distribution of plant population and spacing between plants and rows was as follows. In treatment 1, cabbage monoculture, a spacing of 0.45 x 0.65 m was used, totaling 12 plants per EU. In treatment 2, the combination of cabbage and lettuce, the spacing was 0.45 x 0.65 m for cabbage and 0.25 x 0.35 m for lettuce, totaling 6 cabbages and 20 lettuces per EU. In treatment 3, cabbage was combined with chard, using the same spacing for cabbage (0.45 x 0.65 m) and 0.25 x 0.45 m for chard, resulting in six cabbages and 16 chards per EU. Finally, for treatment 4, which consisted of the association of cabbage and parsley, a spacing of 0.45 x 0.65 m was used for cabbage and 0.125 x 0.25 m for parsley, obtaining six cabbages and 45 parsley plants.

Measurements were taken at 25, 40, and 55 days to determine the percentage weed incidence and cabbage head equatorial and polar diameter were determined at 55 days.

To determine the percentage of incidence of weeds at 25, 40 and 55 days, the random sampling method was adopted where the counting was done through the use of a wooden frame with the dimensions of 1x1 m (Grazziero et al. 2021), the weed count was carried out taking into account all the weeds found in that quadrant, in each part of the planks where the frame was thrown. Scissors, hoes, tape measures, plastic bags and the 1x1 m wooden frame were used for this measurement.

Cabbage yield was determined by weighing approximately 120 harvested cabbage heads using a precision electronic scale and averaging was expressed in tons per hectare. The equatorial and polar diameters of the harvested cabbage heads were measured using a centimeter ruler and averaged results were expressed in centimeters.

The data were subjected to analysis of variance (ANOVA) with the Agrostat® software (Barbosa and Maldonado 2015). In cases where significant differences were detected, mean comparison was performed using the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Percentage of weed incidence

The results shown in Table 1 correspond to the determination of the percentage of weed incidence. It is observed that the lowest incidence rates are exhibited by treatments T1 (cabbage), T2 (cabbage + lettuce), and T3 (cabbage + chard).

Table 1. Mean Comparison for Weed Incidence Percentage (%).

Treatments	Weed Incidence (%)		
	25 days	40 days	55 days
Cabbage + parsley (T4)	75.24^{b}	85.46^{c}	81.00^{b}
Cabbage + chard (T3)	74.30^{b}	52.64^{a}	33.66^{a}
Cabbage (T1)	57.96^{ab}	67.15^{ab}	37.66^{ab}
Cabbage + lettuce (T2)	44.92^{a}	41.00^{a}	30.66^{a}
CV (%)	20.99	19.43	21.42
LSD	22.04	19.90	16.30

Means followed by different letters in the column differ statistically according to the Tukey test at a 5% probability of error, CV=Coefficient of Variance, LSD=Least Significant Difference.

It can be observed that at 25 days, the lowest incidence of weeds was obtained with treatments T1 (cabbage) and T2 (cabbage + lettuce). In contrast, at 40 and 55 days, the lowest results of weed incidence were observed in treatments T1 (cabbage), T2 (cabbage + lettuce) and T3 (cabbage + chard). It should be noted that all the identified weed species, observed both pre- and post-experimentation, are reported to be commonly occurring within the experimental area. The species include *Commelina diffusa* Burm., *Cyperus rotundus* L., *Digitaria ciliaris* (Retz.), *Cenchrus equinatus* L., and *Amaranthus viridis* L., along with the grass *Echinochloa crus-galli* (L.) O. Schulz.

In light of the findings of this determination, it can be posited that the coexistence of two species in a given area gives rise to competitive interactions with weeds, which ultimately prove deleterious to the weeds. Romero et al. (2024) define this phenomenon as phenotypic plasticity, defined as the ability of plants to respond to environmental stimuli with adjustments that generate different phenotypes over time or in different locations, modifying their functional traits. Consequently, plant growth is regulated by external factors, primarily through alterations in morphology, physiology, or biochemistry. In situations of stress, light and water are significant factors that exert a considerable influence on plant growth (Rahn et al. 2018).

As previously stated by Neupane et al. (2021) the practice of intercropping has been demonstrated to reduce the prevalence of weeds in agricultural systems. This is achieved by facilitating the capture of essential nutrients and other accessible resources. The presence of glucosinolate compounds in cruciferous plants has been identified as a contributing factor to weed suppression, due to their allelopathic effects. Furthermore, intercropping allows for the utilization of space and nutrients that would otherwise be unutilized by a single crop. This limits the space available for weed growth and proliferation.

However, to obtain efficient intercropping systems with bio-economic advantages, it is essential to manage the species to be cultivated effectively, as well as to consider production factors such as green manure, population densities of the component crops, and so forth (Lino et al. 2021).

Cabbage Yield

As indicated in Table 2, T2 exhibited superior outcomes in terms of weight, although its results are statistically equivalent to those observed in T1. A comparison of cabbage yield in monoculture and polyculture demonstrated that the crop in the monoculture system produced 36.975 (ton ha^{-1}) and 50,008 (ton ha^{-1}) in association with lettuce. However, statistically significant differences were observed when comparing the cabbage yield in association with lettuce and cabbage with chard and parsley.

Table 2. Mean Comparison for Yield (ton ha^{-1}).

Treatments	Yield (ton ha^{-1})
Cabbage + lettuce (T2)	50.008^{a}
Cabbage (T1)	36.975^{ab}
Cabbage + chard (T3)	34.908^{b}
Cabbage + parsley (T4)	34.208^{b}
CV (%)	24.36
LSD	0.79

Means followed by different letters in the column differ statistically according to the Tukey test at a 5% probability of error, CV=Coefficient of Variance, LSD=Least Significant Difference.

According to Hernández (2019), determining the influence of polyculture on pests and productive indicators in *Brassica oleracea* L., where estimated cabbage yields were above 60 (ton ha^{-1}) in both treatments (monoculture and association). These data were higher than those obtained in this experiment.

It can be stated that the productivity and quality of cabbage heads are influenced by several factors, including plant population, genotype, climatic conditions, water regime and soil nutritional status (Tegen and Jembere 2022). It is thus important to consider that the intercropping species should not impede the growth of the main crop, should not compete with it, and should be a species that is advantageous to the main crop. This was demonstrated in the present investigation, where the combination of cabbage and lettuce resulted in a positive yield outcome. Therefore, when considering methods of increasing yield, cultivation practices such as intercropping are the optimal choice (Turan et al. 2022).

Equatorial Diameter of Cabbage Head

The analysis of variance (ANOVA), as presented in Table 3, revealed no statistically significant differences between the treatments in the determination of the equatorial diameter of the head of cabbage using two production systems (monoculture and association). The resulting data yielded the following measurements: 18.84 cm (T1), 21.18 cm (T2), 18.15 cm (T3), and 17.50 cm (T4).

Table 3. Mean Comparison for Equatorial Head Diameter.

Treatments	Equatorial Head Diameter (cm)
Cabbage + lettuce (T2)	21.18[a]
Cabbage (T1)	18.84[a]
Cabbage + parsley (T4)	18.15[a]
Cabbage + chard (T3)	17.50[a]
CV (%)	24.36
LSD	0.79

Means followed by different letters in the column differ statistically according to the Tukey test at a 5% probability of error, CV=Coefficient of Variance, LSD=Least Significant Difference.

The fact that there were no statistical differences in cabbage head diameter based on the cropping system in this trial may be because cabbage and other leafy green vegetables have different requirements and do not compete for production factors. Head diameter is also considered to be an important component of cabbage yield.

El-Remaly et al. (2022) refer that cabbage is a relatively mid-season crop that grows slowly in the first growth phase, which provides the opportunity to intercrop a short-season crop such as lettuce with the main crop while maintaining yield and quality. Furthermore, lettuce is a short-season crop and its water and mineral requirements do not conflict with those of the main crop, cabbage. This reinforces the fact that there were no differences between the treatments applied in the present investigation.

Llomitoa et al. (2023) observed that the use of dolomite and earthworm humus in the cultivation of cabbage (*Brassica oleracea* L. var. capitata) resulted in head diameter measurements ranging from 19.70 to 22.67 cm, with an average of 20.92 cm. They found no statistical difference between these measurements, indicating that the replications within the experimental unit were properly distributed and homogeneous. These findings align with the results of the present experiment.

Polar Diameter of Cabbage Head

The ratio of the length of the polar and equatorial diameters defines the characteristic shape of the head of a given cabbage cultivar, and both dimensions also determine the weight of the cabbage head. Plant spacing is influential in any cultivation system and cultivar, mainly in cabbage cultivation (Girard and Osorio 1975). No significant differences were found regarding the effect of different planting densities on the variable polar diameter of the cabbage head (Table 4).

In a study of intercropping lettuce and broccoli, Martinez et al. (2024) observed a significant improvement in broccoli yield and suggested that to mitigate nutrient competition in intercropping systems, it is recommended to select plant species with different rooting patterns, nutrient requirements, and periods of peak nutrient demand.

Table 4. Mean Comparison for Polar Head Diameter.

Treatments	Equatorial Head Diameter (cm)
Cabbage + lettuce (T2)	19.25[a]
Cabbage + parsley (T4)	18.71[a]
Cabbage (T1)	17.69[a]
Cabbage + chard	17.48[a]
CV (%)	11.67
LSD	3.55

Means followed by different letters in the column differ statistically according to the Tukey test at a 5% probability of error, CV=Coefficient of Variance, LSD=Least Significant Difference.

In contrast to the findings of the present investigation, Mladenova and Yordanova (2023) who evaluated the effect of intercropping on cabbage growth and yield, concluded that, regardless of small differences, the intercropping option influenced the height and diameter of cabbage heads, indicating that intercropping had the most complex effect on the analyzed indicators, evidencing that when two species are intercropped, the dominant species can increase its yield and nutrient uptake, while the yield of the other crop is reduced due to interspecific competition for nutrients (Shanmugam et al. 2022).

CONCLUSION

This study highlights the importance of intercropping in improving cabbage yield and weed management. Intercropping cabbage with lettuce or chard not only reduced weed incidence, but also resulted in higher yields than monoculture, with the highest yield recorded in treatment T2 (cabbage + lettuce).

Overall, the results support the adoption of intercropping strategies to increase economic viability and promote sustainable agriculture.

It is recommended intercropping systems be implemented to specific conditions and crop combinations to maximize yields and minimize weed competition. In addition, further research should investigate the long-term effects of different intercropping strategies on pest management and soil properties to refine sustainable horticultural practices.

CONFLICT OF INTERESTS

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

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Research article

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Revista Facultad Nacional de Agronomía

Interference of *Conyza sumatrensis* on grain yield of soybean cultivars

UNIVERSIDAD NACIONAL DE COLOMBIA

Interferencia de *Conyza sumatrensis* en la productividad de grano de cultivares de soya

https://doi.org/10.15446/rfnam.v78n1.112580

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ABSTRACT

Sumatran fleabane (*Conyza sumatrensis*) weed can be found in several different agricultural environments and impacts different crops, such as soybean and maize. This weed may have a substantial impact on soybean yield. The aim was to evaluate the interference of *C. sumatrensis* on the grain yield of soybean cultivars. Soybean cultivars were used with late or early maturity, under 0, 1, 2, 3, 4, 6, 8, and 10 plants m^{-2} of *C. sumatrensis*. The four trials, composed of the two cultivars in each of the growing seasons, were analyzed separately. Because differences were found to be significant using the F-test in the comparison between trials ($P \leq 0.05$). The yield was subjected to analysis of variance and F-test. A nonlinear, rectangular hyperbolic regression model was fitted. For the early maturity cultivar, infestation levels of 17.1 and 17 of plants m^{-2} in the 2016–2018 and 2017–2018 growing seasons, respectively, were required to cause a 50% yield loss. For late-maturity cultivars, the values were 6.3 and 7.0 in the 2016–2017 and 2017–2018 growing seasons, respectively. The yield reduction observed for the late-maturity cultivar was 12.54 and 13.72% per plant of *C. sumatrensis,* in the 2016–2017 and 2017–2018 growing seasons, respectively. The early maturity cultivar showed a reduction of 9.35 and 10.77% per plant, in the 2016–2017 and 2017–2018 growing seasons, respectively. *Conyza sumatrensis* that cannot be tolerated in soybean, because a single plant per m^2 has great potential for reducing yield, from 9.35 to 13.72%.

Keywords:
Agronomic performance
Crop-weed competition
Erigeron sumatrensis Retz
Weed

RESUMEN

La maleza rama negra (*Conyza sumatrensis*) se puede encontrar en varios entornos agrícolas diferentes y afecta a diferentes cultivos, como la soja y el maíz. Esta maleza puede tener un impacto sustancial en la productividad de la soja. El objetivo fue evaluar la interferencia de *C. sumatrensis* en la productividad de grano de cultivares de soja. Se utilizaron cultivares de soja con madurez tardía o temprana, bajo 0, 1, 2, 3, 4, 6, 8 y 10 plantas m^{-2} de *C. sumatrensis*. Los cuatro ensayos, compuestos por los dos cultivares en cada una de las cosechas, se analizaron por separado. Debido a que se encontraron diferencias significativas utilizando la prueba F en la comparación entre ensayos ($P \leq 0{,}05$). La productividad fue sometida a análisis de varianza y prueba F. Se ajustó un modelo de regresión no lineal, hiperbólico rectangular. Para el cultivar de madurez temprana, se necesitaron niveles de infestación de 17,1 y 17 plantas m^{-2} en las cosechas 2016–2018 y 2017–2018, respectivamente, para causar una pérdida de productividad del 50%. Para el cultivar de madurez tardía, los valores fueron de 6,3 y 7,0 en las cosechas 2016–2017 y 2017–2018, respectivamente. La reducción de la productividad observada para el cultivar de madurez tardía fue del 12,54 y 13,72% por planta de *C. sumatrensis*, en las cosechas 2016–2017 y 2017–2018, respectivamente. El cultivar de madurez temprana mostró una reducción del 9,35 y 10,77% por planta, en las cosechas 2016–2017 y 2017–2018, respectivamente. *Conyza sumatrensis* no puede tolerarse en la soya, porque una sola planta por m^2 tiene un gran potencial para reducir la productividad, del 9,35 al 13,72%.

Palabras clave:
Desempeño agronómico
Competición entre cultivos y malezas
Erigeron sumatrensis Retz
Maleza

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Weed interference occurs through allelopathy, parasitism, and competition, which can affect crop development and yield (Horvath et al. 2023). This is especially true for the *Conyza* genus, with prolific weeds belonging to the Asteraceae family, as an example, the Sumatran fleabane (*Conyza sumatrensis* (Retz.) E. Walker sin.: *Erigeron sumatrensis* Retz.) (Bajwa et al. 2016). This weed has an annual life cycle, with high seed production, which can easily be dispersed longer distances from the parent plant (Liu et al. 2022). Thus, these plants can be found in several different agricultural environments, affecting crops, such as soybean and maize (Bajwa et al. 2016; Kalsing et al. 2024).

Trezzi et al. (2015) indicated that 2.7 *Conyza bonariensis* plants m^{-2} can reduce soybean yield by 50%. Agostinetto et al. (2017) reported that only one *C. bonariensis* plant m^{-2} can reduce soybean yield by up to 25.9%. Similarly, *Conyza canadensis* can reduce soybean yield by more than 90%, when chemical control measures are not adopted (Byker et al. 2013). In the southern region of Brazil in a subtropical climate, *C. sumatrensis* can reduce soybean grain yield by up to 50% (Blainski et al. 2015). In contrast, *C. sumatrensis* did not interfere with the agronomic performance of soybeans, in a study carried out in the Brazilian Cerrado biome during a hot and rainy summer. Under these conditions, the death of *C. sumatrensis* plants occurred, which can be explained due to shading by the crop (Correia 2023). In Brazil, there is a higher prevalence of *C. sumatrensis* than that of *C. bonariensis* in the southern region (Marochio et al. 2017; Ruiz et al. 2022). Including several reported cases of herbicide resistance for *C. sumatrensis* in this region of the country (Baccin et al. 2022; Heap 2024).

Twenty herbicide-resistant biotypes of *C. sumatrensis* have been reported worldwide (Heap 2024). In Brazil, there are cases of multiple resistance to chlorimuron and glyphosate (Santos et al. 2014), resistance to paraquat (Zobiole et al. 2019), and cases of single or multiple resistance to these and other herbicides (Pinho et al. 2019; Albrecht et al. 2020; Queiroz et al. 2020; Lorenzetti et al. 2024). Cases of herbicide resistance make it difficult to manage *Conyza* spp. and can increase production costs. The cost of managing glyphosate-resistant weeds in maize, cotton, and soybean fields in the United States alone has reached US$1 billion per year (Frisvold et al. 2017). Adegas et al. (2017) found that the control costs for herbicide-resistant *Conyza* spp. in Brazil were approximately 32% higher.

Weed interference studies can also assess the effects of weed-density and weed-crop proportions (Swanton et al. 2015). Weed management-related decisions mainly depend on an economic threshold, originating from the crop and cropping system. Furthermore, manipulation of soybean cultivars or growing seasons can increase crop competitiveness and change weed control decisions (Korres et al. 2020). *Conyza sumatrensis* may have a substantial impact on soybean yield. There are a few specific studies with this weed, which can be contrasting depending on the region of Brazil (Blainski et al. 2015; Correia 2023). Therefore, it is important to provide specific data for *C. sumatrensis*, given the prevalence of this species in Brazil (Ruiz et al. 2022; Kalsing et al. 2024). Thus, the aim was to evaluate the interference of *C. sumatrensis* on the grain yield of soybean cultivars.

MATERIALS AND METHODS

Weed-crop interference

Experiments were conducted in soybean fields at Palotina, Paraná, Brazil, during the 2016–2017 and 2017–2018 growing seasons using two soybean cultivars per season. The soils were very clayey (14% sand, 22% silt, and 64% clay), originated from basalt, and had a pH=5.9 and organic matter of 2.7%. The fields were managed under no-tillage, with a soybean-maize rotation system. The climate of the region is classified as Cfa according to the Köppen, with the weather conditions for the experimental period illustrated in Figure 1.

The cultivars M5947 and M6210 were sown on September 22 and 11, respectively, during the 2016–2017 growing season. In the 2017–2018 growing season, M5947 was sown on September 22 and M6210 on October 4 (following seed companies' recommendations). M6210 (late maturity cultivar - maturity group 6.2) has a longer cycle than M5947 (early maturity cultivar - maturity group 5.9). Both soybean cultivars have indeterminate growth habits. In the 2016-2017 growing season, fertilization was carried out at sowing with 206 kg ha^{-1} of fertilizer 02-20-18 (NPK), for the 2017-2018 growing season, 248 kg ha^{-1} of fertilizer 02-18-18 (NPK) was used. Phytosanitary management was carried out to keep the crop free of biotic factors

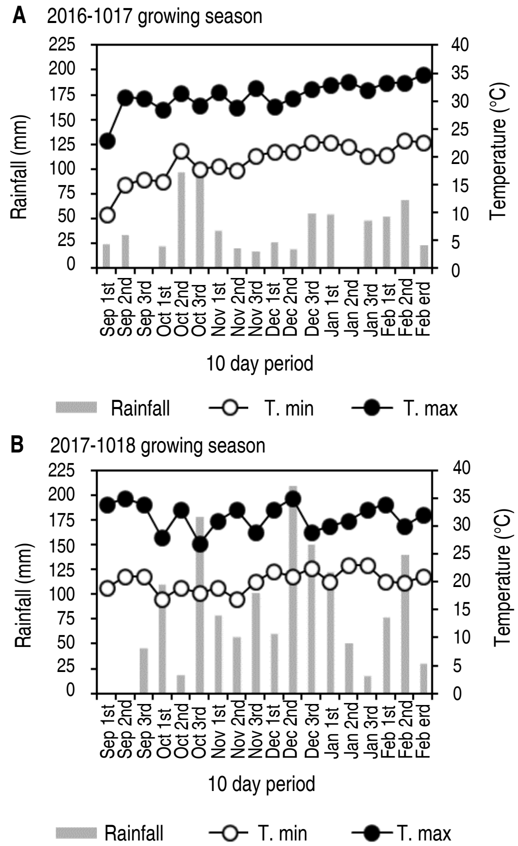

Figure 1. Rainfall, maximum (T. max), and minimum temperature (T. min) during the period of the experiments, 2016-2017 (A) and 2017-2018 (B) growing seasons.

that could interfere with the growth and development of soybean plants, following technical recommendations appropriate for the region.

The experimental design was a randomized complete block design, with three and four repetitions in 2016–2017 and 2017–2018, respectively. The treatments consisted of different densities of *C. sumatrensis*: 0, 1, 2, 3, 4, 6, 8, and 10 plants m^{-2}. The plots consisted of six 5 m long rows of soybean plants spaced at 0.45 m, adding up to 13.5 m^2 for each plot. To obtain the desired populations at the four trials, the plots were completely weeded manually up to 14 days before soybean sowing. *C. sumatrensis* plants that coexisted with soybeans emerged from this date. During soybean sowing, *C. sumatrensis* plants were up to 10 cm tall and had 1 to 3 leaves. From soybean sowing until approximately 40 days after emergence (closure between rows), weeding was carried out to maintain *C. sumatrensis* densities and control weeds of other species. Weeding was carried out once a week. For all four trials, there was a large infestation of *C. sumatrensis* before weeding began (approximately 15 plants m^{-2}). Plants with the best distribution throughout the plot were chosen, and those present in the space between the soybean rows were prioritized. As weeding progressed, new emergence flushes of *C. sumatrensis* were easily identified. Young seedlings were eliminated, while those previously selected were larger. No herbicides were used to control weeds; all control was carried out by hand weeding, to avoid any herbicide injury on soybeans or *C. sumatrensis*.

Furthermore, soybeans were harvested manually at the R_8 stage (full maturity). The plants of the two central rows were harvested at 4 m in length, adding up to 3.6 m^2. The grains produced in each plot were weighed, and the moisture was corrected to 13%. Furthermore, the yield in kg ha^{-1} was calculated using this data.

Analysis of yield loss and critical level of losses

The four trials, composed of the two cultivars in each of the growing seasons, were analyzed separately. Because differences were found to be significant using the F-test in the comparison between trials ($P \leq 0.05$). Yield data were subjected to the analysis of variance by F-test ($P \leq 0.05$) using the Sisvar 5.6 software (Ferreira 2011). A nonlinear, rectangular hyperbola regression model was fitted to the data using SigmaPlot 12 software (Kalsing and Vidal 2013; Machado et al. 2015), following Equation 1:

$$y = \frac{a * x}{b + x} \quad (1)$$

Wherein "y" is equivalent to the normalized data for the yield loss in comparison to the weed-free plots, expressed as a percentage (%); "a" is the maximum asymptote or yield loss when the weed density is close to the carrying capacity of the environment; "b" is the level of infestation that is equivalent to approximately 50% yield reduction, and "x" is the level of infestation. The critical level of damage (i) was then obtained by the ratio between parameters "a" and "b" of the equation, representing the impact of each plant on the crop yield.

RESULTS AND DISCUSSION

Conyza sumatrensis substantially reduced soybean yield even under low densities in both cultivars and seasons. For the early maturity soybean cultivar, infestation levels of 17.1 and 17 (parameter b) of *C. sumatrensis* m^{-2} in the 2016–2017 and 2017–2018 growing seasons, respectively, were required to cause a 50% yield loss of soybean crop. For the late-maturity cultivar, the values were 6.3 and 7.0 in the 2016–2017 and 2017–2018 growing seasons, respectively. In the 2016–2017 growing season, the parameter "i" (proportional yield loss when the weed density approaches zero) was 12.5% for the late-maturing cultivar and 9.35% for the early-maturity cultivar. In the 2017–2018 growing season, the parameter "i" was 13.7% for the late-maturity cultivar, whereas it was 10.77% for the early-maturity cultivar, which was 22% lower compared to the late-maturity cultivar. That is, a single plant of *C. sumatrensis* m^{-2} can reduce soybean yield by up to 13.7% (Table 1).

Table 1. Equation parameters obtained by nonlinear rectangular hyperbole regression, for early and late maturity soybean cultivars and growing season.

Growing season	Cultivar	Equation parameters			
		a	b	i (%)	R^2
2016-2017	Early maturity	159.1±63.2	17.1±9.9	9.35	0.93
	Late maturity	79.1±18.0	6.3±2.9	12.54	0.89
2017-2018	Early maturity	183.2±48.2	17.0±6.3	10.77	0.95
	Late maturity	96.1±6.6	7.0±0.9	13.72	0.98

a: maximum asymptote. b: the value of the level of infestation that equals 50% of yield reduction. i: soybean yield loss per weed unit when its density approaches zero.

Trezzi et al. (2015) indicated that 2.7 of *C. bonariensis* m^{-2} plants could reduce soybean yield by approximately 50%, which is higher than that observed in the present study. Moreover, Agostinetto et al. (2017) found that a single plant m^{-2} of *C. bonariensis* could reduce soybean yield by approximately 25.9%. In contrast, *C. sumatrensis* at densities of 13 to 23 plants m^{-2} did not interfere with the agronomic performance of soybeans, in a study carried out in the Brazilian Cerrado biome during a hot and rainy summer. Under these conditions, the death of *C. sumatrensis* plants occurred, which can be explained due to shading by the crop (Correia 2023). In the southern region of Brazil in a subtropical climate, such as this study, 20 to 35 plants m^{-2} of *C. sumatrensis* can reduce soybean grain yield by up to 50% (Blainski et al. 2015). Weed interference can be highly dependent on the cultivar and weed genotypes and the present environment (Roncatto et al. 2021; Caldas et al. 2023). Therefore, comparative tests can be conducted to determine the tolerance of cultivars at different environments in comparison with the weeds.

The current study identified that for both cultivars, growing seasons, and densities used, *C. sumatrensis* has a substantial impact on soybean yield. There are a few specific studies on *C. sumatrensis*, which can be contrasting depending on the soil and climate conditions (Correia 2023). Therefore, it is important to provide specific data for *C. sumatrensis* in a subtropical climate, given the prevalence of this species in the southern region of Brazil (Marochio et al. 2017; Ruiz et al. 2022), which reiterates the relevance of this study. Other weeds highlighted for their negative impact on soybean yield include *Amaranthus palmeri* (Korres et al. 2019), *Digitaria insularis* (Gazziero et al. 2019), and *Amaranthus tuberculatus* (Butts et al. 2018), which are among the most important weeds in soybean crop.

In the 2016–2017 growing season, the early maturity cultivar had a grain yield in the absence of *C. sumatrensis* was 3,992 kg ha^{-1}. For the highest level of infestation (10 plants m^{-2}) the average yield reduction was 58%, with a grain yield of 1,677 kg ha^{-1}. Moreover, the late-maturing cultivar had a grain yield of 3,861 kg ha^{-1} in the absence of *C. sumatrensis*. The maximum loss (48%) was observed at the highest level of infestation (10 plants m^{-2}), with a grain yield of 2,008 kg ha^{-1} (Figure 2).

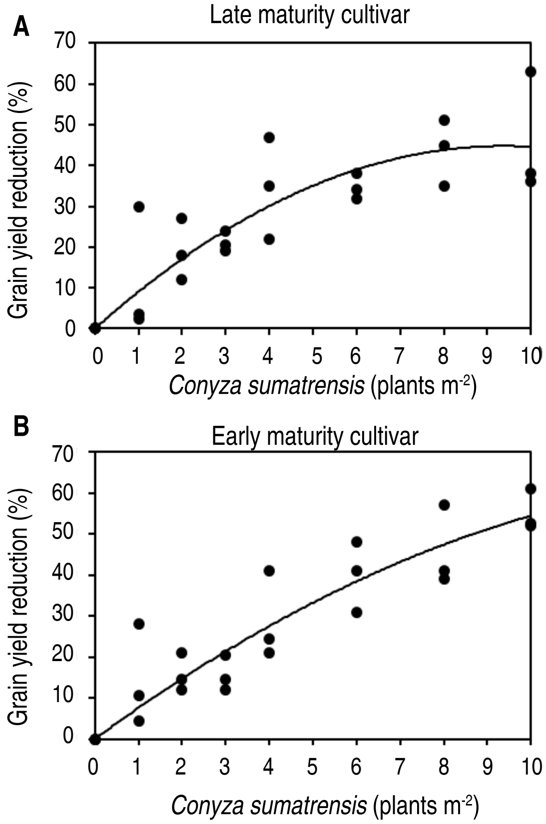

Figure 2. Nonlinear regression of rectangular hyperbole for soybean yield reduction under densities of *C. sumatrensis*. Late (A) and early (B) maturity soybean cultivars, 2016-2017 growing season.

In 2017–2018, the grain yield for the late-maturing cultivar was 4,311 kg ha^{-1} without weed infestation, with maximum loss (57%) for 10 plants m^{-2} with a grain yield of 1,854 kg ha^{-1}. The early maturity cultivar produced 4,019 kg ha^{-1} in the absence of infestation. Under the influence of 10 plants m^{-2} the yield was reduced by 73%, with a grain yield of 1,085 kg ha^{-1} (Figure 3).

At maximum infestation (10 plants m^{-2}), there was a minimum reduction of 48.7% in soybean yield. These results show the substantial influence of *C. sumatrensis* on soybeans, making it necessary to maintain weed density at the lowest level, or absent, because even a single plant of *C. sumatrensis* m^{-2} has a considerable impact on soybean yield, until 13.72%. Trezzi et al. (2015) concluded that a single plant of *C. bonariensis* m^{-2} can reduce soybean grain yield by up to 36%. Competition between *C. bonariensis* and soybeans between 21 and 42 days after crop emergence can represent a reduction of 21 kg ha^{-1} in yield per day of coexistence (Silva et al. 2014). This reinforces the need to keep soybean crops free from the presence of *Conyza* spp.

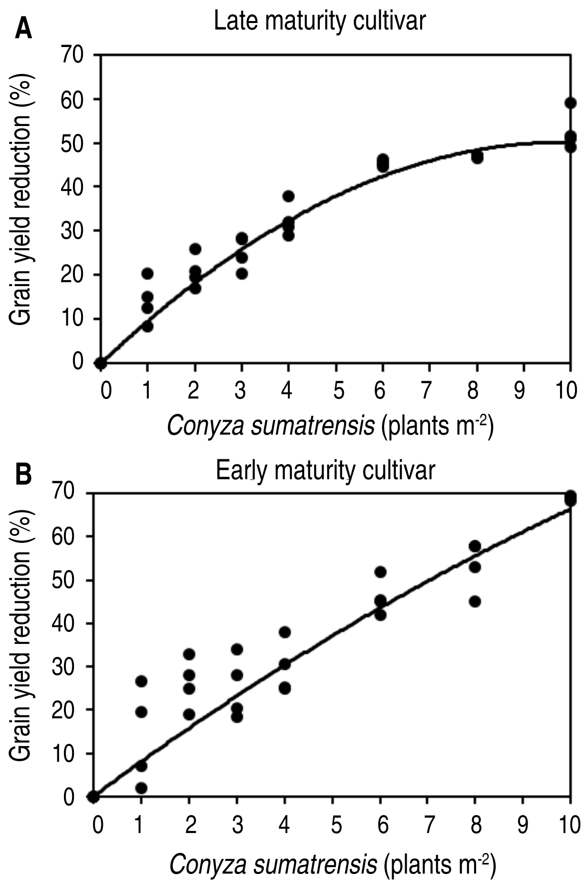

Figure 3. Nonlinear regression of rectangular hyperbole for soybean yield reduction under densities of *C. sumatrensis*. Late (A) and early (B) maturity soybean cultivars, 2017-2018 growing season.

Some characteristics help in explaining the high aggressiveness of *Conyza* spp. plants, including vigorous growth, plasticity in their life cycle, and their ability to adapt to different environments (Bajwa et al. 2016; Baccin et al. 2022). As well as when in competition, it can affect the growth and development of soybean shoots and roots (Rockenbach and Rizzardi 2020). Furthermore, some studies have also indicated the allelopathic effects of *Conyza* spp. on other plant species (Ferreira et al. 2020; Peralta et al. 2022). *Conyza* species were dominant in some environments, with the presence of few weeds of other species (Concenço and Concenço 2016). The ecophysiological characteristics of *Conyza* associated crop management, no-till system, continuous use of herbicides for control, and other aspects have favored the selection of resistant biotypes and dominance of this weed (Bajwa et al. 2016; Baccin et al. 2022). Therefore, this reinforces the competitive ability of this plant, even about other weeds, and helps in explaining the impact of a single m^{-2} plant on soybean yield in the present study. The aggressiveness of *Conyza* spp. restates the importance of effective control, which keeps the population levels of this plant close to zero. In this study, this is reinforced by the data obtained for *C. sumatrensis*. Thus, the adoption of herbicides in pre- or post-emergence in combinations (Cantu et al. 2021; Albrecht et al. 2022; Garcia et al.

2023; Monteiro et al. 2024), cover crops (Wallace et al. 2019; Bunchek et al. 2020; Fisher and Sprague 2022, 2023), and herbicide combinations with cover crops (Schramski et al. 2021) are fundamental for the control of this and other weeds. For example, vetch and barley crop residues were effective in suppressing *C. canadensis* (Campiglia et al. 2015), also maize, *Urochloa*, ryegrass, turnip, wheat, and black oat crop residues were effective in suppressing *C. bonariensis* (Lamego et al. 2013). Which, in the sum of research, highlights the need for integrated weed management.

The adoption of these and other practices for the management of *Conyza* spp. is not only important for controlling and suppressing low population densities but also for controlling the advance of herbicide-resistant biotypes. Therefore, the presence of even a single plant m^{-2} of *C. sumatrensis* should not be tolerated. This condition must be sought in integrated weed management, with the aim of achieving economic sustainability in soybean crops (Bajwa et al. 2016; Riemens et al. 2022). The integration of control methods is important, with preference given to those that reduce the emergence of weeds, such as crop rotation that provides soil cover with crop residues. Hand weeding is very expensive, and it becomes unfeasible even for small areas to control aggressive weeds, estimates indicate that a rural worker needs 15 days to weed one hectare, with successive interventions in order to keep the crop free from weed interference (Van der Weide et al. 2008). The cost of manual weeding can approach US$ 200 ha^{-1}, a high cost per hectare, it was not considered a viable option in the economic analysis (Dominschek et al. 2021).

Thus, the adoption of herbicides in pre- or post-emergence combinations, cover crops, and herbicide combinations with cover crops are fundamental for the control of this and other weeds. However, there are a few studies on *C. sumatrensis*, whereas most of the interference studies have been conducted on other species of *Conyza*. Therefore, extensive research on different production systems and agroecosystems needs to be carried out. Comparative tests can be conducted to determine the tolerance of cultivars in comparison with the weeds.

CONCLUSION

The grain yield reduction observed for the late-maturity cultivar was 12.54 and 13.72% per plant of *C. sumatrensis*, in the 2016–2017 and 2017–2018 growing seasons, respectively. The early maturity cultivar showed a reduction of 9.35 and 10.77% per plant of *C. sumatrensis*, in the 2016–2017 and 2017–2018 growing seasons, respectively. *Conyza sumatrensis* cannot be tolerated in soybean crops, because a single plant per m^2 has great potential for reducing grain yield.

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Effect of hydrothermal processing on the native starches of cassava (*Manihot esculenta*) and yam (*Dioscorea alata*)

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Efecto del proceso hidrotérmico en almidones nativos de yuca (*Manihot esculenta*) y ñame (*Dioscorea alata*)

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ABSTRACT

Keywords:
Gelatinization
Heat-moisture treatment
Retrogradation
Tubers

Currently, there is a need to develop starches with improved properties to enhance their applicability in food matrices. The effect of the hydrothermal treatment (HMT) on the physicochemical, morphological, and structural properties of native cassava and yam starches was evaluated. Native cassava and yam starches were subjected to low moisture (20 - 25%), a high temperature (90 °C), and a processing time of 4 hours. The results showed that HMT significantly decreased in cold water solubility (CWS), in cassava starch while increasing its water absorption capacity (WAC) and degree of crystallinity (DC). In contrast, yam starch displayed the opposite effects. Furthermore, the modification increased amylose content and paste stability. Additionally, microphotography revealed significant changes in granular morphology. In conclusion, hydrothermal treatment of tuber starches is a promising technology for improving the hydrophilic properties and pasting characteristics of cassava and yam starches, supporting the development of clean-label products.

RESUMEN

Palabras clave:
Gelatinización
Tratamiento calor-humedad
Retrogradación
Tubérculos

En la actualidad, surge la necesidad de desarrollar almidones con propiedades mejoradas para aumentar su aplicabilidad en matrices alimentarias. Se evaluó el efecto del tratamiento hidrotérmico (HMT) sobre las propiedades fisicoquímicas, morfológicas y estructurales de los almidones nativos de yuca y ñame. Los almidones nativos de yuca y ñame se sometieron a baja humedad (20 - 25%), alta temperatura (90 °C) y un tiempo de procesamiento de 4 horas. Los resultados mostraron que el HMT disminuía significativamente la solubilidad en agua fría (CWS), al tiempo que aumentaba la capacidad de absorción de agua (WAC) y el grado de cristalinidad (DC) de los almidones de yuca. En cambio, en el ñame se produjo el efecto contrario. Además, la modificación aumentó el contenido de amilosa y la estabilidad de las pastas. Adicionalmente la microfotografía reveló cambios significativos en la morfología granular. En conclusión, el tratamiento hidrotérmico en almidones de tubérculos es una tecnología prometedora para mejorar las propiedades hidrofílicas y las características de empastamiento de los almidones de yuca y ñame, garantizando el desarrollo de productos de etiqueta limpia.

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Starch is a polysaccharide deposited in birefringent semicrystalline aggregates that are insoluble in cold water and serve as the main source of energy storage (Bernal et al. 2022). This polymer is of great interest across various industries due to its versatility as a thickening, gelling, and texturizing agent. However, native starch exhibits functional limitations in its physicochemical and morphological properties, including low shear resistance, insolubility in cold water, thermal degradation, and a high level of retrogradation, which restrict its use in diverse products (Barragán et al. 2016). These limitations can be addressed by employing new technologies to enhance desirable properties or inhibit certain undesirable properties of native starch.

As a result, various methods—physical, chemical, and enzymatic—have been applied to modify starches and increase their industrial utility. Although chemical modification methods are widely used in industry, there is growing concern about their environmental impact, driving researchers to explore innovative, eco-friendly alternatives rooted in clean technologies (Maniglia et al. 2021). For this reason, physical modification methods have gained attention, as they are more environmentally friendly and can improve the intrinsic properties of starch while preserving the general structure of the granules without inducing gelatinization, a key factor in industrial applications (Sobowale et al. 2022; Solarte-Montúfar et al. 2019).

In this context, heat-moisture treatment (HMT) is regarded as one of the most environmentally friendly and low-cost alternative technologies. HMT enhances the physicochemical, rheological, and structural properties of starches without altering granular morphology (Klein et al. 2013; Yeh and Lai 2021). Recent studies have shown that HMT applied to tuber starches increases thermal stability, affects gelatinization temperatures, and introduces variations in the semicrystalline arrangement of starch granules (Barua et al. 2021; Dudu et al. 2020; Jia et al. 2022).

Given the limitations of native tuber starches, this study proposes the implementation of physical modification processes, such as hydrothermal treatment, to develop starches with enhanced hydrophilic capacity and high resistance to thermal stress. This research aims to determine the impact of heat-moisture treatment on the physicochemical, morphological, and structural properties of native cassava and yam starches as a technological alternative in developing innovative and eco-friendly starch modification processes for tuber-derived materials.

MATERIALS AND METHODS

Native cassava starch (NCS), variety "M-TAI" was supplied by the company Almidones de Sucre S.A.S. The yam "Criollo" was purchased at the local market in Sincelejo, Sucre, and native yam starch (NYS) was extracted in the unit operations plant of the Universidad de Sucre, following the methodology proposed by Salcedo-Mendoza et al. (2018) with some modifications. Specifically, chemical extraction (ammonia solution) was replaced by physical processes, and centrifugation washes were implemented to remove the mucilaginous material. Standards for potato amylose (A0512, USA) and corn amylopectin (10120, USA) were acquired from Sigma-Aldrich®. All reagents used in the research were of analytical grade.

Modification by heat-moisture treatment (HMT)

Cassava and yam starches were hydrothermally modified following the method proposed by Klein et al. (2013), with slight modifications. Initially, 30 g samples (d.b.) were hydrated to a moisture content of 20 and 25% (w/w), and then stored at room temperature for 24 h to ensure homogenization. Subsequently, the hydrated samples were subjected to 90 °C for 4 h in a forced convection oven (FD115, BINDER, Germany).

Amylose content and molecular order by FTIR-ATR

Amylose Content and Molecular Order by FTIR-ATR. The amylose content was estimated following the methodology proposed by Seña-Rambauth et al. (2024). FTIR-ATR spectra were recorded from 500 to 4,000 cm^{-1} with 32 scans at a resolution of 4 cm^{-1} using a Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (Spectrum, PerkinElmer, USA). The degree of molecular order (OM) was calculated as the absorbance ratio in the bands 1,047/1,022 cm^{-1} and 995/1,022, expressed as a percentage according to de Dios-Avila et al. (2022).

Diffraction pattern (XRD) and relative degree of crystallinity (DC)

The XRD was estimated using an X-ray diffractometer (Panalytical, X'Pert MPD, Switzerland). Spectra were acquired over a range of 4-40 °, operating at 1.8 kW and 40 mA (Davoudi et al. 2022). The relative degree of crystallinity (DC) was determined by calculating the ratio of the absorption peaks in the crystalline regions to the total area, using MATLAB for data processing.

Morphological characteristics

The morphology and birefringence capacity of the starch granules were examined using a trinocular polarized light microscope with bright field capabilities (Optika, B-383POL, Italy) (Seña-Rambauth et al. 2024).

Physicochemical properties

Water absorption capacity (WAC) was analyzed following the method proposed by Cao and Gao (2019). Cold water solubility (CWS) was determined using the method proposed by Salcedo-Mendoza et al. (2018), with slight modifications. For this, 0.125 g of starch was weighed and mixed with 12.5 mL of distilled water, homogenized for 5 min and centrifuged at 1,701 x g for 15 min. Cold water solubility (%) was calculated by weight difference and estimated based on mass gain. Swelling power (SP) was analyzed according to the methodology of Li et al. (2011) with slight modifications. A 0.5 g starch sample was added to 12.5 mL of distilled water preheated to 60 °C. Samples were placed in a water bath at 60 °C for cassava starch and 70 °C for yam starch for 30 min. After this time, they were centrifuged at 1,701 x g for 15 min. The supernatant was discarded, and the weight of the tube containing the gel was recorded. The weight of the gel and the amount (g) of dry solids recovered by evaporation were determined.

Pasting and gelatinization properties

Pasting properties were determined using the methodology proposed by Ramos-Villacob et al. (2023). Gelatinization properties were assessed through temperature scanning using a rheometer (Anton Paar MCR 302, Austria) using parallel plate geometry. Initially, the linear viscoelastic region was determined by performing an amplitude sweep. Subsequently, a temperature sweep was conducted between 30 and 90 °C at a constant frequency of 1 Hz and a strain of 0.5%. (Cham and Suwannaporn 2010). The values of the storage modulus (G′ [Pa]) and the loss modulus (G″ [Pa]) were obtained during the process

Experimental design and statistical analysis

A categorical one-factor design was established with six levels corresponding to the type of starch, as described in Table 1 The results were analyzed using analysis of variance (ANOVA) and Tukey's test for mean comparison at a significance level of 5%, using the statistical software Statgraphics (Centurion XVI, Statgraphics Inc., version XVI, USA).

Table 1. Experimental design implemented in the hydrothermal modification of starches.

Treatment	Nomenclature
Native cassava starch	NCS
Cassava starch with 20% w/w moisture content, modified by HMT	SCH-20
Cassava starch with 25% w/w moisture content, modified by HMT	SCH-25
Native yam starch	NYS
Yam starch with 20% w/w moisture content, modified by HMT	SYH-20
Yam starch with 25% w/w moisture content, modified by HMT	SYH-25

RESULTS AND DISCUSSION

Amylose content (AC)

The CA results for cassava and yam starches are presented in Table 2. The CA of NCS ranged between 18 and 20%, differing from the 24% reported for cassava starch by Ramos-Villacob et al. (2023). This difference is likely associated with cassava variety and crop conditions, such as harvest times and weather. The CA of NYS fluctuated between 24 and 25%, similar to the result obtained for yam starch by Salcedo-Mendoza et al. (2018).

After treatment, CA increased significantly in the modified starches (P<0.05), except for the SHY-25 treatment, which did not show significant differences compared to its native counterpart. Additionally, it was observed that the HMT treatment conducted at 20% moisture induced higher CA in cassava and yam starches (Table 2). These results are consistent with those reported for cassava starch treated with HMT by Liu et al. (2016). The authors suggest that such changes may be associated with chain disorder and a reduction in amylose leaching. They also reported that the degradation of amylopectin during HMT could explain the increase in amylose content after HMT treatment.

Table 2. Percentage of amylose content, relative degree of crystallinity and molecular order in native and modified starches.

Treatment	Amylose (%)	OM_1 (%)	OM_2 (%)	DC (%)
NCS	19.45±0.45[a]	0.737±0.00[ac]	1.184±0.02[a]	47.46±0.53[a]
SCH-20	22.94±0.09[b]	0.695±0.03[b]	1.233±0.03[bc]	49.56±0.46[b]
SCH-25	21.84±0.11[c]	0.727±0.00[ba]	1.202±0.02[ab]	50.65±0.41[b]
NYS	24.94±0.26[d]	0.749±0.00[ac]	1.281±0.00[c]	45.10±0.30[c]
SYH-20	25.60±0.12[e]	0.755±0.00[ac]	1.337±0.04[c]	42.80±0.08[d]
SYH-25	24.94±0.41[d]	0.777±0.02[c]	1.281±0.00[c]	40.50±0.45[e]

AC: amylose content; DC: relative degree of crystallinity; NCS: native cassava starch; SCH-20: cassava starch with 20% w/w moisture content, modified by HMT; SCH-25: cassava starch with 25% w/w moisture content, modified by HMT; NYS: native yam starch; SYH-20: yam starch with 20% w/w moisture content, modified by HMT; SYH-25: yam starch with 25% w/w moisture content, modified by HMT; MO_1: estimated molecular order between bands 1,047/1,022; MO_2: estimated molecular order between bands 995/1,022. Means with different lowercase letters in the same column indicate statistically significant differences according to Tukey's test ($P \leq 0.05$).

Furthermore, Liu et al. (2021) argue that HMT may cause thermal degradation of the outer linear chains of amylopectin, which could convert into amylose chains, forming complexes with other amylose chains or lipids. This would account for the increase in amylose content following hydrothermal treatment. Additionally, this effect may be due to less steric hindrance near the α-(1,6) glycosidic bond compared to the α-(1,4) bond, as the HMT treatment effectively broke the α-(1,6) glycosidic bond and disrupted the side chain of amylopectin, thereby producing short-chain amylose.

Molecular Order (MO)

The absorption peaks in the 1,200 to 900 cm^{-1} regions of the FTIR spectra are shown in Table 2. These spectra have been attributed to three main vibrational zones with maximum absorbance at 1,047, 1,022 and 995 cm^{-1}, providing insights into the structural organization of the chains, crystallinity, and retrogradation of starch (Varatharajan et al. 2010; Zheng et al. 2023). The ratio between the bands at 1,047/1,022 cm^{-1} corresponds to ordered and amorphous domains and was used to quantify the degree of molecular order (MO_1) in the starch granules (Table 2). Native cassava and yam starches after HMT showed similar vibrations in the absorption peaks corresponding to the C-H, C-O and O-H groups, possibly associated with the formation of new bonds during molecular reorganization (Mina et al. 2013). The MO_1 values in the cassava starches after the HMT treatment (SCH-20) showed a significant decrease (P<0.05) compared to native starch, which may be attributed to the dissociation of double helices in the crystalline lamellae that form the crystalline matrix of the starch granules (Yu et al. 2021) . In contrast, the MO_1 values of yam starches did not show significant changes (P<0.05) compared to the native counterpart. This indicates that the characteristic peaks of the treatments did not show a representative shift, and no new absorption peaks appeared or disappeared, suggesting that no chemical bonds or functional groups were formed or broken during hydrothermal treatment (Batista et al. 2023).

On the other hand, the ratio between the sharp peaks 995/1,022 cm^{-1} primarily results from the bending vibrations of the C-O-H group, which are sensitive to water content and reflect the water-starch interaction (Yu et al. 2021). This ratio was used to determine the degree of molecular order (MO_2) in the starch granules (Table 2). After hydrothermal treatment, the OM_2 value in cassava starch significantly increased (P<0.05) in the SCH-20 treatment compared to the native counterpart. This result is consistent with an increase reported by Batista et al. (2023) in breadfruit starch treated with hydrothermal treatment (annealing). The author suggests that the treatment promoted the unraveling and dissociation of the double helix within the crystallites, likely due to increased water content associated with the growth of amorphous regions in the granule. Meanwhile, OM_2 in SCH-25 and SYH-25 did not show significant differences (P<0.05). These variations in molecular order values may be attributed to differences in amylose content, as previously observed.

X-ray diffraction (XRD) and relative degree of crystallinity (DC)

The diffraction patterns of cassava and yam starches are shown in the diffractograms in Figure 1. The results indicate that NCS exhibited characteristic crystalline peaks of A-type polymorphism (Figure 1A), consistent with those reported for native cassava starch by Ramos-Villacob et al. (2023). In contrast, NYS showed a B-type pattern (Figure 1B), similar to yam starch reported by Meaño-Correa et al. (2015). After treatment, the cassava and yam starches showed angles similar to those of the native samples, indicating that the A-type and B-type crystalline structures of the treated samples were not modified.

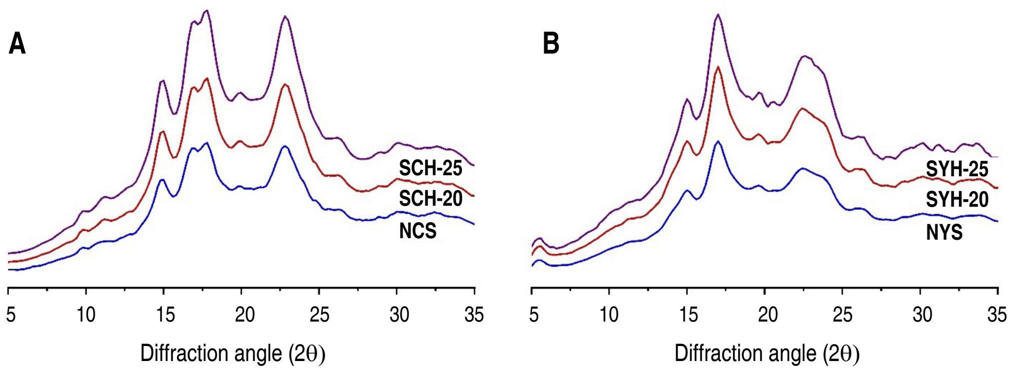

Figure 1. X-ray diffraction patterns (XRD) of. A) native and modified cassava starches, B) native and modified yam starches. NCS: native cassava starch; SCH-20: cassava starch with 20% w/w moisture content, modified by HMT; SCH-25: cassava starch with 25% w/w moisture content, modified by HMT; NYS: native yam starch; SYH-20: yam starch with 20% w/w moisture content, modified by HMT; SYH-25: yam starch with 25% w/w moisture content, modified by HMT.

The effect of HMT on DC is presented in Table 2. NCS showed a DC of 47.46%, consistent with findings reported by Figueroa-Flórez et al. (2019). After HMT, a significant increase (P<0.05) was observed in SCH-20 and SCH-25. Similar results were reported in cassava starch treated with HMT by Moraes et al. (2014), who argued that HMT leads to a reorganization of starch molecules, enhancing molecular interactions and thereby increasing crystallinity (Van et al. 2017). Meanwhile, NYS presented DC similar to that reported for yam starch (Arroyo-Dagobeth et al. 2023). After HMT, a decrease in DC was observed, proportional to the increase in moisture content in the SYH-20 and SYH-25 treatments. This decrease in DC is more evident at higher moisture levels, as water may contribute to the disruption of intra- and intermolecular hydrogen bonds within the starch granule (Liu et al. 2016).

Morphological characteristics

The morphological characteristics of native and modified starches from cassava and yam can be visualized through the analysis of bright-field and polarized light micrographs (Figure 2A, B). Native cassava starch granules exhibited a semi-spherical morphology with a smooth surface, free of pores, and truncated ends. These characteristics are possibly associated with the extraction process, during which starch granules may suffer lacerations due to handling and the physical conditions to which they are subjected, such as grating or crushing the cassava (Figueroa-Flórez et al. 2023). The granules starch had an average granular size of 10.5 μm, similar to that reported for cassava starch extracted from *Manihot esculenta cranz* by Ramos-Villacob et al. (2023).

Native cassava starches, after undergoing hydrothermal treatment, did not exhibit any morphological damage to the granules. However, the HMT treatment led to granular agglomeration, likely due to partial swelling and breakage of the starch granules in the presence of water molecules (Asranudin et al. 2023). Additionally, the treatment caused a 3.5% increase in granular size. These results are consistent with those reported by Moraes et al. (2014) for cassava starch granules, which maintained their morphology after being subjected to hydrothermal treatment under conditions of 30-35% moisture and 90 °C.

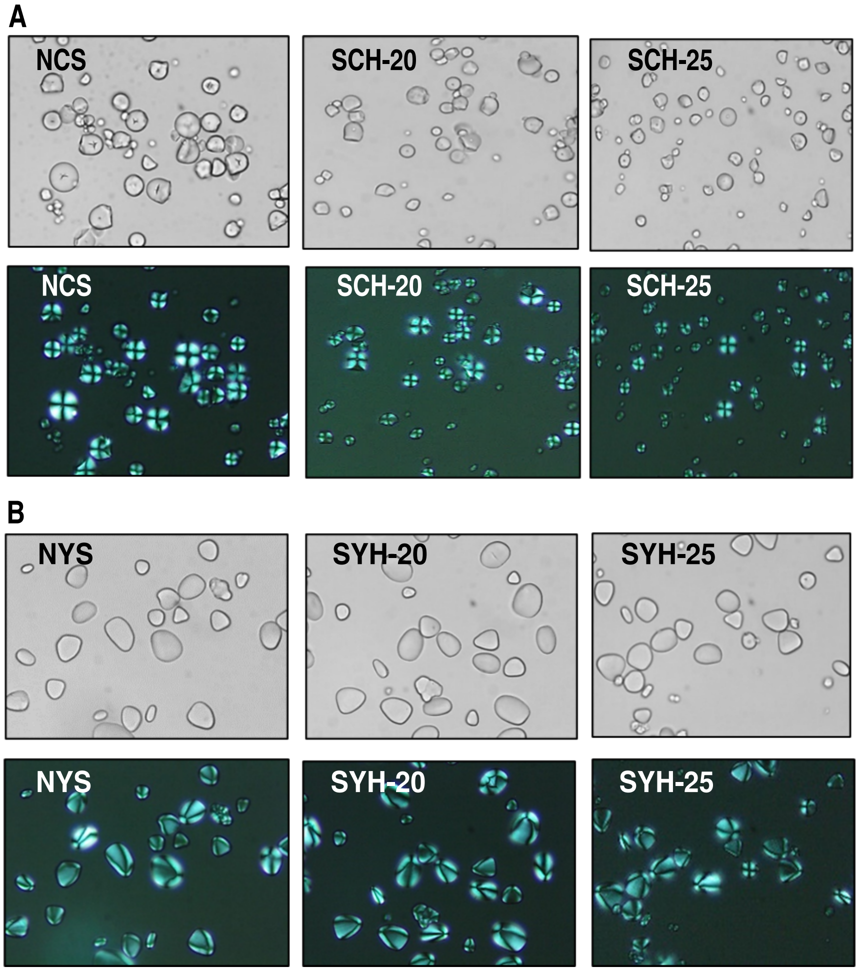

Figure 2. Bright-field and polarized light micrographs of. A) native and modified cassava starches, B) native and modified yam starches. NCS: native cassava starch; SCH-20: cassava starch with 20% w/w moisture content, modified by HMT; SCH-25: cassava starch with 25% w/w moisture content, modified by HMT; NYS: native yam starch; SYH-20: yam starch with 20% w/w moisture content, modified by HMT; SYH-25: yam starch with 25% w/w moisture content, modified by HMT.

The native yam starch granules (NYS) exhibited an oval morphology with a smooth, pore-free surface and an average particle size of 21 μm (Figure 2A). Similar results have been reported by Meaño et al. (2016). Both native and modified starches displayed a maltase cross extending towards the periphery of the granule, with characteristic birefringence typical of starch granules exhibiting a B-type diffraction pattern (Arroyo-Dagobeth et al. 2023). Additionally, the orientation suggests that the position of the 'hilum' is decentralized, which is characteristic of starch granules derived from rhizomes (Chakraborty et al. 2021).

After HMT treatment the morphology of the yam starch granules was preserved, with the presence of granular agglomerations similar to those observed in yam starch treated at 25% moisture and 110 °C, as reported by Indrianti and Pranoto (2018). An increase in granule size was also noted. Chen et al. (2022) suggest that this increase could be attributed to the thermal energy released during HMT, which may cause migration within the starch chain.

Physicochemical properties

(CWS), and swelling power (SP) of cassava and yam starches are shown in Table 3. NCS exhibited a WAC in the range of 70–75%, similar to results reported for cassava starch by Salcedo-Mendoza et al. (2017). After HMT treatment, cassava starches showed higher WAC (*P*<0.05), consistent with results reported by Liu et al. (2016) for HMT-treated starch. These authors suggest that such changes in WAC are likely associated with stronger interactions between hydroxyl and water molecules in HMT-treated samples compared to their native counterparts. NYS exhibited WAC in the range of 95–98%, consistent with values reported by Otegbayo et al. (2011) for yam starch. After HMT, yam starches showed a significant decrease (*P*<0.05) in WAC (Table 3).

Some studies have linked the reduction in WAC and swelling of starch granules to potential intragranular molecular reorganization, which reduces water accessibility to amorphous regions. This result aligns with the decrease in DC, as the lower WAC observed after HMT is attributed to the denser crystalline structure, which limits the starch's capacity to absorb water and swell (Liu et al. 2016; Oyeyinka et al. 2021).

Table 3. Physicochemical properties of native cassava and yam starches and HMT-treated starches.

Treatment	WAC (%)	SP(g/g)	CWS (%)
NCS	71.51±0.95[a]	2.95±0.15[a]	4.06±0.06[a]
SCH-20	75.83±0.43[b]	2.12±0.04[b]	3.68±0.04[ba]
SCH-25	76.83±0.81[b]	2.28±0.09[b]	3.65±0.04[b]
NYS	97.62±0.42[c]	2.16±0.02[b]	5.31±0.24[c]
SYH-20	93.37±0.28[d]	2.17±0.01[b]	4.60±0.25[d]
SYH-25	94.48±0.16[e]	2.16±0.02[b]	4.78±0.03[d]

WAC: Water absorption capacity; CWS: Solubility in cold water. SP: Swelling power. TG: Granule size. NCS: native cassava starch; SCH-20: cassava starch with 20% w/w moisture content, modified by HMT; SCH-25: cassava starch with 25% w/w moisture content, modified by HMT; NYS: native yam starch; SYH-20: yam starch with 20% w/w moisture content, modified by HMT; SYH-25: yam starch with 25% w/w moisture content, modified by HMT. Means with different lowercase letters in the same column indicate statistically significant differences according to Tukey's test ($P \leq 0.05$).

Native cassava starch exhibited SP values between 2.0 and 4.0 aligning with the ranges reported for various cassava varieties (Figueroa-Flórez et al. 2019). Following HMT, the SP of cassava starches decreased significantly (*P*<0.05), consistent with findings by Moraes et al. (2014) for HMT-modified cassava starch. This decrease in SP may be attributed to a loss of starch granule integrity upon high swelling, as the treatment could increase crystallinity, reduce starch hydration, and enhance interactions between amylose and amylopectin molecules (Sobowale et al. 2022; Suriya et al. 2019).

In contrast, native yam starch showed SP values ranging from 2.1 to 2.3%, consistent with those reported for *Dioscorea bulbifera L.* starch by Meaño et al. (2018). The HMT-modified yam starch did not show significant differences (*P*<0.05) compared to the native starch. This suggests that hydrothermal treatment did not affect the granular morphology of yam starch, resulting in limited granule swelling. This finding is supported by the micrographs (Figure 2B) in this study, which indicate that the structure and size of the granules remained similar to those of the native starch.

The cold-water solubility (CWS) of native cassava and yam starches was within the ranges reported for cassava starch by Figueroa-Flórez et al. (2023) and yam starch by Arroyo-Dagobeth et al. (2023). Following treatment at varying moisture levels, the CWS of cassava and yam starches decreased significantly (*P*<0.05). These results align with the study on HMT-treated yam starch conducted by Suriya et al. (2019), which suggests that a decrease in CWS may result from increased interactions between amylose-amylose and amylopectin-amylopectin chains due to heat treatment.

Pasting properties

The viscosity behaviors of native and modified starches are presented in Table 4. The NYS treatment showed a significantly higher (*P*<0.05) pasting temperature (TP) compared to NCS. A higher TP suggests greater internal stability of the starch granule, an increased presence of semicrystalline regions, and is generally associated with a higher amylose content (Salgado et al. 2019). Regarding viscosities, NCS exhibited a higher peak viscosity (PV) than NYS. Additionally, the BV values indicated greater stability for the NYS treatment, while the SV was higher in NYS compared to the NCS treatment. Similar findings on cassava and yam starches were reported by Arroyo-Dagobeth et al. (2023), who concluded that differences in these starchy materials are influenced by crystalline pattern type, as starches with a B-type diffraction pattern exhibit greater retrogradation compared to those with an A-type pattern (Klein et al. 2013).

Table 4. Pasting profiles of native cassava and yam starches and HMT-treated starches.

Treatment	PT (°C)	PV (Cp)	BV (Cp)	SV (Cp)
NCS	68.17±0.25[a]	2688±1.15[a]	935.7±19.0[a]	31.7±18.5[a]
SCH-20	70.43±0.15[b]	2574±1.15[b]	26.7±6.11[b]	364±4.16[b]
SCH-25	74.40±0.20[c]	2263±1.00[c]	2.0±1.00[b]	193±2.89[c]
NYS	82.80±0.20[d]	1724±1.53[d]	286±34[c]	1439±32.0[d]
SYH-20	87.53±0.76[e]	159.1±8.31[e]	125±6.54[d]	1020±2.08[e]
SYH-25	87.80±0.20[e]	183.1±4.96[f]	129±0.47[d]	671±6.12[f]

PT: Pasting temperature; PV: Peak viscosity; BV: Breakthrough viscosity; SV: Settling viscosity. NCS: native cassava starch; SCH-20: cassava starch with 20% w/w moisture content, modified by HMT; SCH-25: cassava starch with 25% w/w moisture content, modified by HMT; NYS: native yam starch; SYH-20: yam starch with 20% w/w moisture content, modified by HMT; SYH-25: yam starch with 25% w/w moisture content, modified by HMT. Means with different lowercase letters in the same column indicate statistically significant differences according to Tukey's test ($P \leq 0.05$).

After HMT treatment, the pasting temperature (PT) significantly increased (*P*<0.05) in both cassava and yam starches. This result may be linked to increased cross-linking of starch chains within the granular matrix, which requires more heat for structural degradation and gel formation. The peak viscosity (PV) of HMT-treated cassava and yam starch granules was significantly lower than that of the native controls (Table 4). Similar results have been reported in the literature for HMT-treated wheat starch by Liu et al. (2015), who suggested that the decrease in PV could be due to the loss of some branched chains during the modification process, leading to less swelling of the starch granules during heating (Li et al. 2017).

Conversely, cassava and yam starches exhibited a significant decrease (*P*<0.05) in breakdown viscosity (BV) after HMT treatment compared to their native counterparts. A similar decrease was observed by Indrianti and Pranoto (2018) in HMT-treated sweet potato starch, who argued that the reduction in BV enhances paste stability and could be associated with a decrease in swelling power. This is likely due to the strengthening of the gel matrix structure and amylose, which improves stability during shearing (Chung et al. 2009). Additionally, this decrease may result from associations between chains in the amorphous regions of the granule and changes in crystallinity during hydrothermal treatment (Trung et al. 2017).

In contrast, modified cassava starches showed an increase in setback viscosity (SV), indicating a greater tendency for retrogradation due to increased suspension viscosity during cooling (Paternina et al. 2016; Suriya et al. 2019). Meanwhile, modified yam starches exhibited a significant decrease (*P*<0.05) in SV compared to the native starch. This decrease in viscosity could be associated with an increased amylose-to-amylopectin ratio; starches with higher amylose content are likely to exhibit greater structural stability due to the stronger attraction between polymeric chains (Solarte-Montúfar et al. 2019).

Gelatinization properties

In this study, oscillatory dynamic tests were conducted to establish the gelatinization temperatures (Table 5). The results showed that elastic moduli (G') predominated over viscous moduli (G''), suggesting that the starch hydrogels exhibit an elastic structure (Glorio et al. 2009). The thermal effect in the range of 20 to 90 °C can disrupt the short-range double helices formed between amylopectin/amylopectin or amylopectin/amylose complexes, defining the boundaries of swelling and granular gelatinization processes. The gelatinization temperatures obtained align with those estimated by Barua et al. (2022) and Van et al. (2017) for native cassava and yam starches. From these findings, it can be inferred that rheological testing with a temperature sweep within the linear viscoelastic region is an effective method for determining gelatinization properties (Seña-Rambauth et al. 2024).

The gelatinization temperatures of native and modified starches are presented in Table 5. Initially, native yam starch (NYS) exhibited higher gelatinization temperatures than native cassava starch (NCS). These results are consistent with studies on cassava and yam starch reported by Van et al. (2017) and Martínez et al. (2019). These temperature differences are likely related to the crystalline pattern of each starchy material. NYS exhibits a B-type pattern, which contains abundant amylopectin chains with a high degree of polymerization, potentially requiring greater thermal energy to initiate gelatinization in NYS compared to NCS (Klein et al. 2013).

Table 5. Gelatinization properties estimated by oscillatory temperature sweeps on native and HMT-treated starches.

Treatment	To (°C)	Tp (°C)	Tc (°C)	Tc- To (°C)
NCS	61.74±0.01[a]	68.83±0.00[a]	75.94±0.02[a]	14.95±0.01[a]
SCH-20	62.72±0.03[b]	70.85±0.04[b]	77.99±0.00[b]	15.27±0.03[b]
SCH-25	63.75±0.00[c]	73.92±0.00[c]	79.40±0.00[c]	15.65±0.00[c]
NYS	74.91±0.00[d]	82.04±0.00[d]	86.13±0.00[d]	11.22±0.00[d]
SYH-20	83.07±0.01[e]	86.05±0.08[e]	90.17±0.00[e]	7.1±0.01[e]
SYH-25	86.11±0.00[f]	88.14±0.00[f]	90.20±0.00[e]	4.09±0.00[f]

To: initial temperature; Tp: peak gelatinization temperature; T_c: final temperature. NCS: native cassava starch; SCH-20: cassava starch with 20% w/w moisture content, modified by HMT; SCH-25: cassava starch with 25% w/w moisture content, modified by HMT; NYS: native yam starch; SYH-20: yam starch with 20% w/w moisture content, modified by HMT; SYH-25: yam starch with 25% w/w moisture content, modified by HMT. Means with different lowercase letters in the same column indicate statistically significant differences according to Tukey's test ($P<0.05$).

Following HMT treatment, the gelatinization temperatures (To, Tp, Tc) of cassava and yam starches significantly increased ($P<0.05$) compared to their native counterparts. The increase in gelatinization temperatures of the modified starch granules could be attributed to structural changes within the starch granules, such as the destabilization of crystalline regions and the leaching of amylose chains (Sun et al. 2014).

The differential temperature (Tc-To) in HMT-treated cassava starches (SCH-20 and SCH-25) increased significantly ($P<0.05$), while it decreased in other HMT-treated starches. This increase in differential temperature likely reflects the degree of heterogeneity among crystallites within the starch granules (Pardo et al. 2013). This property varies based on amylose content, as well as the size, shape, and distribution of the starch granules, and their internal interactions (Liu et al. 2021). Furthermore, changes in the thermal properties of cassava starch granules may be attributed to the formation of amylose-lipid inclusion complexes and enhanced amylose-amylopectin interactions during hydrothermal treatment. These interactions could lead to a reduction in amorphous regions and an increase in melting temperatures (Liu et al. 2016; Xie et al. 2019).

CONCLUSION

Hydrothermal treatment (HMT) facilitated the development of modified cassava and yam starches, resulting in structural alterations without significant changes in granular morphology compared to native starches. In cassava starch, HMT led to an increase in amylose content and molecular ordering, enhancing cold water solubility and reducing breakdown viscosity. Similarly, HMT in yam starch increased amylose content, altering the semicrystalline order of the granules and significantly affecting hydrophilic properties and pasting viscosities. Furthermore, hydrothermal treatment at 25% moisture (HMT) modified the hydrophilic properties, resulting in starches with stable breakdown viscosities and a lower tendency to retrogradation. Thus, hydrothermally treated starches could be suitable for use in baked goods or pastries. However, further studies are recommended, such as evaluating resistant starch or slow-digesting starch content through simple hydrothermal processes or repeated cycles.

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Microencapsulation of non-polar extracts of Colombian propolis via spray drying

Microencapsulación de extractos apolares de propóleos mediante secado por aspersión

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ABSTRACT

Keywords:
Antioxidant activity
Antimicrobials
Bioactivity
Propolis
Spray drying

The oily extracts of propolis are matrices with a high antiradical, reducing, and antimicrobial properties. However, because of their susceptibility to oxidizing agents (oxygen and light) that react with the environment and negatively affect bioactivity and sensory properties, their use remains limited. Oily extracts of Colombian propolis (OECP) were microencapsulated (MOECP) by spray drying using maltodextrin and Gum arabic as the wall material and the conservation of active principles and the control of the release of bioactive components were evaluated. The formulations of MOECP with a higher concentration of propolis extract and lower drying temperature exhibited higher bioactivity because of less degradation of metabolites and less production of prooxidant substances. The samples exhibited a high antioxidant, antiradical, and antimicrobial potential. However, polyphenols and carotenoids were lost. Mixtures of maltodextrin and Gum arabic were suitable for microencapsulation and more than 50% of the phenols and carotenoids in MOECP were preserved.

RESUMEN

Palabras clave:
Actividad antioxidante
Antimicrobianos
Bioactividad
Propóleos
Secado por aspersión

Los extractos oleosos de propóleos se han caracterizado por ser matrices con un alto poder antirradical, reductor y antimicrobiano. Sin embargo, debido a su susceptibilidad a los agentes oxidantes (Oxigeno y luz) que reaccionan con el medio ambiente y afectan negativamente su bioactividad y propiedades sensoriales han limitado su uso. Para preservar la protección de los principios activos y permitir la liberación controlada de sus componentes bioactivos, se evaluó la microencapsulación de extracto oleoso de propóleos proveniente del bosque húmedo premontano colombiano (EOPC) por medio de secado por aspersión (Spray Drying). Para tal fin fueron utilizados como materiales de pared maltodextrina y goma arábiga. Los EOPC microencapsulados (EOPCM) fueron analizados mediante análisis fisicoquímicos y microbiológicos. Los resultados indican que los EOPCM presentaron un alto potencial antioxidante, antiradical y antimicrobiano y las formulaciones con mayor concentración de EOPC y menor temperatura de secado tuvieron mayor bioactividad debido a una menor degradación de metabolitos y a la menor producción de sustancias pro-oxidantes. Se concluye que es posible elaborar EOPCM usando mezclas de maltodextrina y goma arábiga y se conserva más del 50% de los fenoles y carotenoides en la muestra del EOPC.

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Propolis is an oily and resinous mixture naturally produced by bees (*Apis mellifera*) that isolates and protects hives from temperature changes, mechanical forces, and harmful biological vectors, while also providing collective social immunity at the colony level (Pasupuleti et al. 2017). This mixture is based on botanical materials collected by bees. Because the plant material has contact with the digestive system of bees before being incorporated into the hive compartments, propolis are considered an animal-based product (Seven et al. 2018). The profile of bioactive propolis molecules varies according to geographic and botanical origin, time of year, type of collection, bee genetics, and environmental factors (Gomes do Nascimento et al. 2019). For example, oily extracts are obtained from various bioactive compounds, such as polyphenols, carotenoids, terpenes, esters, amino acids, vitamins, minerals, and sugars (Bankova et al. 2014; Ahangari and Naseri 2018), that have antipathogenic properties both in vitro and *in vivo* (Šuran et al. 2021).

Recent studies have demonstrated that oily extracts of propolis (OEP) are rich in hydrophobic compounds like terpenoids, fatty acids, and waxes, which exhibit superior antimicrobial activity compared to hydrophilic fractions pointing their potential as a source of natural bioactives for diverse applications (Altabbal et al. 2023).

The aqueous and ethanolic extracts of propolis are more common; however, few studies have examined at the properties of oily propolis extracts in terms of their chemical composition and biological activity. EOPs contain nonpolar substances, such as essential oils, fatty acids, lipids conjugated with phenolic compounds, waxes, and resins, which are bioactive compounds of interest due to their antioxidant, antiradical, and antimicrobial activity (Ramanauskienė and Inkėnienė 2011). The antimicrobial potential of EOPs can exceed the bactericidal capacity of propolis extracts rich in polar substances (Almuhayawi 2020). However, the chemical constituents of the essential oil fraction in propolis are prone to degradation, mainly by oxidative agents such as light, pH, and oxygen (Pant et al. 2022). Consequently, encapsulation processes are needed to prevent chemical reactions, preserve bioactive compounds, and maintain biological activity for use as a food additive.

The microencapsulation process creates a microparticle of defined morphology with marked stability by wetting a mixture of bioactive compounds with a biopolymer solution (Kashif et al. 2022). Processes can be based on physical principles such as spray drying or chemical processes such as coacervation and the formation of β-cyclodextrin complexes, depending on many factors such as concentration, temperature, pH, chemical nature, and protective substances (Brahmi et al. 2021). Aspects such as the microparticle morphology and yield, which vary depending on the method and protocol, must be considered to reduce the loss of active ingredients and optimize the process to obtain a high content of biocompounds and enhance bioactivity for a desirable product (Đordevic et al. 2014).

In the microencapsulation process, the compounds used for the microcapsule wall must be determined to protect the substances, avoid unwanted reactions between the bioactive compounds and the medium, and avoid alterations to the organoleptic properties and degradation of organic compounds. The effects of temperature and pH changes also be studied. Microencapsulation improves the physical and rheological properties of material by increasing their solubility and facilitating their handling and incorporation, resulting in sensory defects, and increased bioactive compound activity (McClements et al. 2007).

The Spray drying method is an encapsulation process that forms a powder via atomization and continuous drying under hot air from micro drops, forming a high-quality powder with low water activity that protects the active material (Fernandes M et al. 2014).

The wall materials determine the effectiveness of the process and thus guarantee the stability of the final product. Two of the most widely used materials in microencapsulation processes are maltodextrin and Gum arabic. Maltodextrin is a low-cost polymer with a neutral flavor, low viscosity, and good protective capacity but poor emulsifying capacity. It must be supplemented with another biopolymer, such as Gum arabic, that can integrate with maltodextrin because of their similar properties, and has a greater emulsifying capacity. The use of biopolymers such as maltodextrin and Gum arabic in microencapsulation processes, particularly through spray drying, has proven effective in protecting sensitive bioactive compounds and preserving their functional properties. The appropriate ratio of these biopolymers is crucial to minimizing the loss of non-polar compounds in oily propolis extracts (OPE), such as fatty acids, waxes, and resins, which significantly contribute to their antioxidant and antimicrobial activity (Brahmi et al. 2021). Studies have reported successful encapsulation of extracts and oils from various plants, including sunflower, soybean, and flaxseed (Carneiro et al. 2013). However, due to the novel of this matrix, an oily extract from Colombian beekeeping propolis has not been encapsulated. In this work, we considered the hypothesis that microencapsulation with Gum arabic and maltodextrin can preserve the bioactive properties of oily extracts of propolis.

The increasing demand for natural alternatives to synthetic antibiotics and preservatives highlights the importance of exploring bioactive compounds with antioxidant and antimicrobial properties. These compounds have potential applications in agri-food, cosmetic, and pharmaceutical industries, driving innovation in product development. Therefore, this study aimed to encapsulate oily extracts from propolis obtained in a Colombian premontane humid forest (OECP), using a combination of maltodextrin/gum arabic as wall materials, followed by physicochemical and microbiological characterizations.

MATERIALS AND METHODS

Propolis was supplied by Campo Colombia S.A.S, and obtained from a Colombian premontane humid forest (1,000–2,000 meters above sea level (masl), Latitude: 4.33323, Longitude: -75.8283, 4°19′60″ North, 75°49′42″ West, 18–24 °C) and the bee species *Apis mellifera*. Chemical reagents, such as dichloromethane: ethanol: water and β-carotene standard, were obtained from Sigma Aldrich®. Maltodextrin and Gum arabic were purchased from Cimpa® SAS. The reagents used for the microbiological analyses (Mueller Hinton® culture medium and microdilution plates) were obtained from Elementos Químicos Ltd.

OECP produced using organic solvents

To obtain OECPs, a mixture of non-polar and polar solvents was used, which allowed the effective extraction of total lipids, following the methodology of Kubiliene et al. (2015). 75 mL of dichloromethane: ethanol: water (1:1:1) were mixed with 25 g of bee propolis, which were shaken for 15 minutes at 4,000 rpm in an Eppendorf® 5427 R centrifuge. Three ultrasound cycles were applied at 60 Kw for 15 min at room temperature while protected from light exposure. Subsequently, the sample was filtered using Whatman® Grade 1:11 µm paper. The filtrate was subjected to funnel decantation to isolate the organic phase from the aqueous phase. The apolar region was subjected to another separation to remove waxes. Finally, the organic phase was rota evaporated at 40 °C in a Büchi® R-215 Rotavapor System to remove dichloromethane.

Microencapsulation of OECPs and characterization of microencapsulated (MOECP)

The OECPs were microencapsulated using the methodology proposed by Busch et al. (2017) with some modifications. Maltodextrin (30 g) and 0 Gum arabic (0.3 g) were used, dissolved in 150 mL of distilled water, maintaining a constant ratio between the solvent and the encapsulating agent following the formulations proposed in Table 1. Ten milliliters of oily propolis extracts were added, and the resulting mixtures were homogenized using an Ultra-Turrax T25: IKA®, Germany, at 15,000 rpm for 2 min. Then, the emulsion was dried, and the oily propolis extracts were encapsulated using a Büchi® B-191 Mini Spray Dryer. The spray drying operating conditions were suction (%) = 85; pump (%) = 10; air and feed flow: 0.60 m^3 min^{-1} and 1.3 mm diameter of the nozzle or injector. Yield was calculated according to Equation 1. Samples of microencapsulated OECPs under this methodology were called MOECP.

$$\text{Yield (\%)} = (Mm /(Me)+(Ms)) \times 100 \qquad (1)$$

Where: "Mm" microencapsulated = Mass obtained in the encapsulation, "Me" encapsulant = Mass of the encapsulating agent "Ms" total solids = Mass of the total solids content of the propolis essential oil.

The water activity (A_w) of the MOECP was analyzed by placing the samples in a Rotronic® (HygroLab C1 model), and measurements were performed in triplicate at room temperature.

Table 1. Formulation of microencapsulation from two ratios of encapsulating agent and OECP, and parameters of microencapsulation.

Sample	Encapsulant/OECP ratio	Encapsulation temperature (°C)	Mass of OECP (g)
1	1:0.5	100	15.15
2	1:0.5	120	15.15
3	1:0.5	140	15.15
4	1:0.5	160	15.15
5	1:0.33	100	10.10
6	1:0.33	120	10.10
7	1:0.33	140	10.10
8	1:0.33	160	10.10

OECP: Oily extract of Colombian propolis.
Encapsulant: mixture of maltodextrin and Gum arabic.

Finally, color analysis of MOECP was performed using a Minolta® colorimeter (CM-3600), the CIE-Lab system (L*, a*, b*), illuminant D65 and an observation angle of 10°. The readings were performed in triplicate according to the methodology proposed by López-Patiño et al. (2021).

Table 1 lists the parameters used to describe the microencapsulation process using the Spray Dryer. Eight mixtures, two extract/encapsulant ratios, four temperatures, and two amounts of oily Colombian propolis extract (OECP) were used.

Total content of Phenols and β-carotene of OECP and MOECP

To evaluate the total content of phenols and β-carotene in the OECP and MOECP, the Folin-Ciocalteu method and the β-carotene test were used, respectively. First, pretreatment was carried out with 1:3 dilutions (extract/solvent) for both OECP and MOECP. For 0.5 mL of OECP and 0.5 mg of MOECP, the samples were diluted in 1.5 mL of dichloromethane.

Folin-Ciocalteu method was used to measure the phenol content. A standard curve was prepared from a stock solution of gallic acid in methanol at a concentration of 1 mg mL^{-1}. Once the standard curve was constructed, mixtures of 100 µL of control, 100 µL of OECP and 100 µL of diluted MOECP were made, with a 75 µL of Folin-Ciocalteu solution and 825 µL of 2% sodium carbonate solution. The mixtures were vortexed for 1 min, and 250 µL were poured into each well, repeating this process three times for each sample and control. The plate containing the microdilutions was then stored for 90 min in the dark, and, finally, the mixtures were read at 750 nm in a SpectraMax 190 Microplate Reader® plate reader according to the method proposed by Tiveron et al. (2016).

To measure β-carotene, a calibration curve was prepared from a stock solution made with a β-carotene standard (Sigma Aldrich®) whose concentration was 1 mg β-carotene mL^{-1} of solvent. Subsequently, 250 µL of the diluted OECP and MOECP samples were read at 470 nm using a SpectraMax 190 Microplate Reader® according to the method proposed by Nair and Meliani (2018).

Antiradical activity of OECP and MOECP determined using 1,1-diphenyl-2-picryl hydrazyl (DPPH) method

The DPPH method was used to obtain antiradical activity. A mixture of 0.0079 g of DPPH (Sigma Aldrich®) in 2.5 mL of ethanol was prepared and stirred in an amber bottle for 5 min at room temperature. Subsequently, 250 µL of this solution were diluted in 50 mL of 80% ethanol. Next, 300 µL of this mixture were used for a reading at 540 nm in a SpectraMax® 190 Microplate Reader to verify absorbance ranges between 0.5 and 0.6. Finally, 290 µL of the DPPH solution were mixed with 10 µL of diluted OECP or MOECP, respectively. The plate was left in the dark for 30 min, and the sample was read at 540 nm following the method proposed by Ramadan et al. (2012). The antiradical potential was obtained using Equation 2:

$$\text{DPPH (\%)} = \text{(Absorbance Blank-Absorbance Sample)} / \text{(Absorbance Blank)} \times 100 \quad (2)$$

Iron Reducing Antioxidant Power (FRAP) assay of OECPs and MOECPs

First, the calibration curve was prepared using a standard of ferrous sulfate diluted in 2 mM ferrous sulfate stock aqueous solution. The OECP and MOECP were diluted in dichloromethane at a 1:4 ratio. Subsequently, 90 µL of each dilution was diluted with 270 µL of distilled water in the dark, and then 2.7 mL of a 10 mM TPTZ Sigma Aldrich® solution was added. The mixtures were heated in a water bath at 37 °C for 30 min and then allowed to cool. Finally, absorbance was measured at 595 nm using a SpectraMax® 190 Microplate Reader. The tests were in triplicate, and the concentration was obtained using the slope and cut-off values, which were expressed in µM of ferrous sulfate, in 1 g of propolis following the method proposed by Thaipong et al. (2006).

Analysis of MOECP with Fourier Transform Infrared Spectroscopy (FT-IR)

To identify the functional groups, present in the MOECP compounds, infrared spectroscopy was used to obtain vibration bands characteristic of one or more specific functional groups. For the test, 0.1 g of each sample was weighed, and ground in an FT/IR-4700 FTIR Spectrometer, JASCO®. The readings were taken in the range of 400-4,000 cm^{-1}, in triplicate for each sample. Baseline corrections were made to eliminated outlier values and CO_2 and H_2O bands were removed. The spectra were plotted as a function of wavelength and transmittance percentage. To identify the functional groups of the spectra, the band values were compared with the bibliographic information using the method proposed by Fangio et al. (2018).

Microbiological analysis with minimum inhibitory concentration (MIC) of OECP and MOECP

The antimicrobial activity analysis was performed using the well microdilution technique. Plates with 24 wells were used, and 95 µL of Mueller Hinton® culture medium were added to each plate. Then, 0.05 mL of the OECP for the control treatment and dilutions of MOECP in dichloromethane (1:3) were added to achieve a final concentration in each well of 20 mg mL^{-1}. Once this concentration was reached, 95 µL of Mueller Hinton® medium was added, and serial dilutions were made. Subsequently, a solution of bacteria mix (*Escherichia, Staphylococcus aureus, Salmonella enteritidis, Enterobacter aerogenes, Enterobacter agglomerans, Klebsiella* sp.) was prepared with 100 µL of saline solution at a concentration of 100 µg mL^{-1} and a colony of the bacterial solution with absorbance values between 0.08 to 0.13. Twenty microliters of this solution were added to the wells. Finally, the solutions containing bacteria and OECP or MOECP were incubated for 24 h at 35 °C, and the minimum inhibitory concentration against the microorganisms was determined according to the method proposed by Bonou et al. (2016).

Scanning Electron Microscopy (SEM) of MOECP

The morphology of MOECPs where observed by using scanning electron microscopy (SEM). The tests were performed at the Laboratorio de Microscopía Electrónica de Barrido in the Universidad Nacional de Colombia. The samples were treated with a gold-palladium metallic coating applied with a Q150R ES metallizer (Quorum). Subsequently, they were observed using a Quanta 200 FEI® - USA scanning electron microscope. The operating conditions were at 2×10^{-2} torr and 25.0 kV, using secondary electrons. The aim was to determine the presence of agglomerations, the size of the particles, and the shape and presence of porous structures in MOECP according to the method suggested by Sanchez-Reinoso et al (2017).

Differential Scanning Calorimetry (DSC) of MOECP

DSC analysis was used to determine the enthalpy, glass transition temperatures, and endothermic and exothermic events of the MOECP during the useful life of the samples. A DSC 1-500/2722 Mettler Toledo® calorimetric analyzer was used with 2 mg of each sample placed in an aluminum capsule under N_2, which was supplied at a rate of 50 mL min^{-1}. The temperature range was 30-400 °C, and the heating rate was 10 K min^{-1}. Finally, thermograms were obtained and analyzed using Mettler Toledo® STARe Thermal Analysis System version 8, as proposed by Busch et al. (2017).

Statistical analysis

For each variable, the means and standard deviations were obtained. To analyze significant differences, ANOVA was performed using the Tukey test ($P \leq 0.05$). A principal component analysis (PCA) was also carried out to group the values of the analyses according to similarities and to determinate which variables had a higher incidence in the OECP or MOECP. Matlab® version 7.12.0.635 and Origin® 2018 64 Bit were used (Woźniak et al. 2019).

RESULTS AND DISCUSSION

OECPs extraction

The propolis used in this study were selected because it inhibits coliform microorganisms. Extraction was carried out on eight samples from premontane humid forests, the yield results are presented in Table 2.

Table 2. OECP yields obtained from the propolis collected during the study.

OECP Sample	Mass of propolis (g)	Mass of OECP (g)	Yield (%)
1	25.01	4.87	19.47±0.23[a]
2	25.14	4.95	19.69±0.60[a]
3	24.97	4.76	19.06±010[a]
4	25.03	4.94	19.74±0.57[a]
5	24.89	4.68	18.80±0.46[a]
6	24.96	4.88	19.55±0.52[a]
7	25.04	4.97	19.85±0.39[a]
8	25.01	4.83	19.31±0.28[a]

Different letters in the same column indicate significant differences ($P<0.05$). OECP: Oily extract of Colombian propolis.
Samples 1 to 8 = OECP: Colombian propolis oily extract.

There were no significant differences between the propolis samples, and the yield percentage ranged between 19.06 and 19.85%. This consistency suggests that propolis collected from similar ecological zones under uniform conditions maintains a stable extraction efficiency with apolar organic solvents. Although the yield of the oily extracts was higher than that of essential oils, - had a maximum yield of 1.14%, it was lower than that of the ethanolic extracts, which was between 41 and 60%. Although propolis substances are lipophilic and have a greater affinity with apolar organic solvents, some factors explain the decrease in extraction -relative to that of ethanolic extracts (Sambou et al. 2020). First is that a large proportion of the hydrophobic compounds in propolis are resins (50-70%), whereas oils and waxes comprise 30 and 50% of the total composition (Abdelrazeg et al. 2020). Resins and waxes were removed during the filtration steps; therefore, only oils and substances contained within these matrices will remain (Ahangari and Naseri 2018). The literature reports 41 to 60%, the yields in this study were around 19%. According to Pobiega et al. (2019), water has little affinity to most propolis compounds. Yields with water ranged from 4 to 14%, which affected the results. Although water helped remove waxes and resins, facilitating precipitation, it resulted in a low yield. However, yields increased with ultrasound extraction techniques, which increased the concentration of secondary metabolites and the total mass of the extract. Although OECP yields are modest, the extracted components retain significant biological activity, emphasizing the potential of optimizing extraction techniques, particularly those incorporating ultrasound or alternative solvents, to maximize the recovery of bioactive compounds.

Microencapsulation of OECPs and their characterization (MOECP)

Table 3 presents the results of the yield and aqueous activity of MOECP.

The MOECP exhibited an aqueous activity that ranged from 0.2 to 0.3, which prevented the proliferation of pathogenic fungi and/or bacteria. A significant difference ($P<0.05$) was observed between the samples according to their concentration. The relationship between encapsulant concentration and the water activity was inversely proportional (the higher the concentration of the encapsulant, the lower the water activity). The yields ranged between 35 and 63%, with a higher value observed in samples that had a high concentration of OECP, such as formulations 1 and 2.

The observed inverse relationship between water activity and the concentration of encapsulating agents, such as maltodextrin and Gum arabic, aligns with findings from other studies. Recently, Iesa et al. (2023) indicated that a higher proportion of encapsulants reduces water activity by limiting free water within the microencapsulate matrix, enhancing both stability and microbial inhibition.

Table 3. Performance and aqueous activity of MOECP using different parameters.

Sample MOECP	Encapsulant/OECP ratio	Temperature (°C)	Encapsulant mass (g)	Yield (%)	Aqueous Activity (A_w)
1	1:0.5	100	18.92	62.44	0.31±0.06[a]
2	1:0.5	120	18.32	60.46	0.29±0.07[a]
3	1:0.5	140	15.71	45.25	0.28±0.07[a]
4	1:0.5	160	11.94	39.40	0.27±0.04[a]
5	1:0.33	100	12.90	42.58	0.28±0.05[a]
6	1:0.33	120	11.83	39.04	0.25±0.02[b]
7	1:0.33	140	11.06	36.50	0.23±0.03[b]
8	1:0.33	160	10.85	35.81	0.22±0.04[b]

MOECP: Microencapsulated oily extract of Colombian propolis.
Encapsulant: mixture of maltodextrin and Gum arabic.
OECP: Colombian propolis oily extract.

Moreover, the efficiency of microencapsulation can be influenced by the ratio of encapsulating agents and processing conditions, such as temperature, which affects the retention of bioactive compounds and the uniformity of the microcapsules.

The results of the colorimetry analysis for MOECP are presented in Table 4. The samples had a similar tone (h), without differences (*P*>0.05), but varied in terms of chroma (C) (*P*<0.05). This result was due to changes in luminosity and the blue-yellow ratio when the OECP concentration and temperature changed. As the temperature decreased and the concentration of the encapsulating material increased, the intensity of yellow in the samples increased, and the microencapsulation became less luminous. In the eight formulations, there was a significant difference between to the amount of OECP used and the temperature.

Table 4. OECP and MOECP color parameters.

Sample	L*	a*	b*	C	h
OECP	96.71±3.41[a]	0.17±0.02[a]	4.27±0.30[a]	4.27±0.43[a]	1.53±0.19[a]
1	91.65±2.59[a]	0.64±0.06[a]	5.37±0.41[b]	12.77±0.76[b]	1.54±0.21[a]
2	88.54±3.78[a]	0.66±0.09[a]	5.97±0,57[b]	12.01±0.80[b]	1.45±0.11[a]
3	90.19±4.12[a]	0.87±0.08[b]	6.13±0.39[c]	11.80±0.71[b]	1.43±0.15[a]
4	88.28±4.98[a]	0.86±0.10[b]	6.73±0.48[c]	11.40±0.63[b]	1.44±0.20[a]
5	95.98±4.21[b]	1.09±0.06[b]	11.39±0.76[d]	6.79±0.45[c]	1.48±0.12[a]
6	94.30±3.45[b]	1.67±0.06[c]	10.27±0.69[d]	6.19±0.37[c]	1.41±0.21[a]
7	95.05±7.3[b]	2.28±0.06[d]	11.58±0.81[d]	5.41±0.39[d]	1.38±0.14[a]
8	93.10±5.26[b]	3.44±0.06[c]	11.39±1.09[d]	5.09±0.28[c]	1.35±0.16[a]

Different letters in the same column indicate significant differences (*P*<0.05).
OECP: Oily extract of Colombian propolis.
Samples 1 - 8 = MOECP: Microencapsulated oily extract of Colombian propolis.

The concentrations of bioactive compounds, such as polyphenols and β-carotene, as well as antioxidant and antiradical activity, were reduced in samples 1 to 8 (MOECP), compared with OECP. This phenomenon can be attributed to increased temperature. The most affected metabolites were β-carotene, where the concentration was reduced by up to nine-fold. This can be attributed to the breaking of the double bonds between carotenoids and xanthophylls with increasing temperature, which generated chemical changes in the carotenoids resulting in the appearance of low molecular weight substances, short-chain isoprene derivatives, and oxygenated substances.

Similarly, a decrease in polyphenols was observed with increasing temperature, which could change the original structure and functionality. Phenols react in the presence of substances such as organic acids, lipids, and nitrogenous compounds, giving rise to the appearance of substances such as benzocaine, ethers, glycolipids, aldehydes, and ketones, which have a lower antioxidant capacity than polyphenolic substances or compounds with - OH (Islam et al. 2014; Kalušević et al. 2017).

Total content of Phenols, β-carotene and Antiradical activity of OECP and MOECP

The antioxidant activity, and bioactive compounds content, such as polyphenols and carotenoids in OECP and MOECP are presented in Table 5. Antioxidant activity decreases with increase temperature, leading to the degradation of labile compounds.

Table 5. Antioxidant activity and polyphenol and carotenoid content of OECP and MOECP.

Sample	DPPH (% Inactivation)	mg gallic acid g^{-1} propolis	β-Carotene (μg g^{-1})	FRAP (μmol g^{-1} propolis)
OECP	75.94±3.47^{e}	280.99±4.18^{d}	9.93±0.19^{e}	571.88±21.67^{e}
1	59.09±2.40^{a}	200.12±8.51^{a}	3.55±0.06^{a}	300.25±5.01^{a}
2	57.94±3.47^{a}	180.99±4.18^{a}	2.93±0.19^{a}	318.90±21.67^{a}
3	46.12±3.73^{a}	158.10±15.09^{b}	2.21±0.53^{a}	145.05±4.35^{b}
4	43.80±4.60^{b}	154.52±5.43^{b}	1.95±0.23^{b}	117.72±19.89^{b}
5	29.09±2.40^{c}	100.12±8.51^{b}	1.55±0.06^{b}	200.25±5.01^{c}
6	27.94±3.47^{c}	70.99±4.18^{c}	0.93±0.19^{c}	118.90±21.67^{b}
7	16.12±3.73^{c}	58.10±15.09^{c}	0.21±0.53^{d}	95.05±4.35^{d}
8	13.80±4.60^{d}	54.52±5.43^{c}	0.15±0.23^{d}	67.72±19.89^{d}

Different letters in the same column indicate significant differences ($P<0.05$). OECP: Oily extract of Colombian propolis. Samples 1 - 8 = MOECP: Microencapsulated oily extract of Colombian propolis. DPPH= 1,1-diphenyl-2-picryl hydrazyl. FRAP= Ferric Reduction Antioxidant Power.

Formulations 1 and 2 maintained greater reducing capacity and antiradical activity, indicating less degradation of labile compounds, because they were prepared at lower temperatures. As the temperature increases, Maillard and Strecker degradation reactions generate substances with a lower antioxidant potential than bioactive compounds in OECP. The affected compounds, apart from polyphenols, are low molecular weight volatiles and nitrogenous substances, such as proteins, amino acids, and sugars that result in aldehydes, ketones, pyrazines, and furans. In this study, the values of antioxidant activity and bioactive compounds such as carotenoids were still present in the microencapsulated extracts (MOECP) although at a lower concentration than in the non-encapsulated extract (OECP). On the contrary, previous studies that evaluated the ethanolic extracts of propolis (Baysan et al. 2018) and encapsulated fruit extracts reported better antioxidant activity in encapsulated forms than in non-encapsulated extracts (Tamanna et al. 2015).

Iron Reduction Antioxidant Power Assay (FRAP) for OECP and MOECP

When analyzing the FRAP values for the OECP and MOECP (Table 5), the reducing capacity of the MOECP decrease with increasing temperature and decreasing OECP concentration. According to Rodrigues et al. (2021), microencapsulation reduces the levels of agents in propolis, such as polyphenols, via oxidative processes. Previous studies have shown that microencapsulating has a maximum reducing capacity at temperatures between 100 - 120 °C and that these encapsulates have a greater reducing capacity than encapsulates that only contain maltodextrin and Gum arabic (Pratami et al. 2020). Both polysaccharides and polyphenols have cyclic structures that reduce metal ions such as Fe^{+3}, which is transformed into Fe^{+2}. There are more phytochemical compounds in propolis extracts that contribute to the reducing activity, but they have not been correlated with the reducing power (Pratami et al. 2020).

Analysis of MOECP using FT-IR

Figure 1 shows the results of the infrared spectra reading with the MOECP treatments, which resulted from breaking the double bonds of the carotenoids and xanthophylls as the temperature increased, causing chemical changes in the carotenoids that resulted in the appearance of low molecular weight substances, short-chain isoprene derivatives, and oxygenated substances.

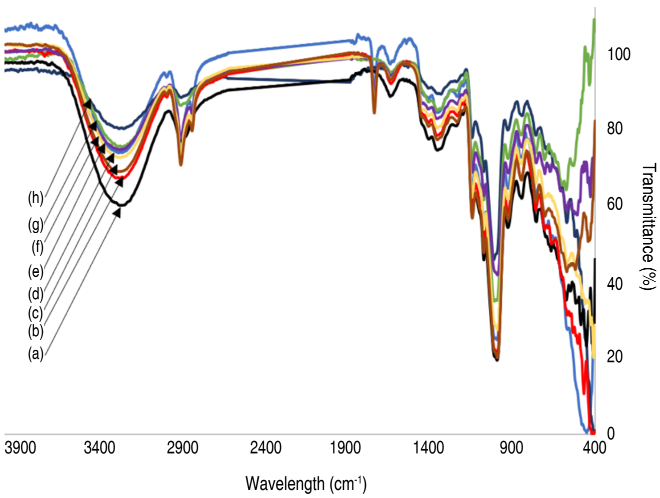

Figure 1. FT-IR spectra of MOECPs. (a) Sample 1 (100 °C), (b) Sample 2 (120 °C), (c) Sample 3 (140 °C), (d) Sample 4 (160 °C), (e) Sample 5 (100 °C), (f) Sample 6 (120 °C), (g) Sample 7 (140 °C), (h) Sample 8 (160 °C). MOECP: Microencapsulated oily extract of Colombian propolis.

The results of the FT-IR spectra suggest that as the temperature increased, there was a noticeable degradation of carotenoids, leading to the formation of low molecular weight substances, short-chain isoprene derivatives, and oxygenated compounds. This thermal degradation is characterized by the disappearance of characteristic absorption bands associated with carotenoid double bonds and the emergence of new peaks corresponding to these degradation products. Such findings are consistent with studies that have observed similar thermal-induced changes in carotenoid structures, which are known to contribute to the aroma profiles of various foods and natural products (Stutz et al. 2015). Additionally, the appearance of oxygenated compounds in the FT-IR spectra suggests oxidative processes occurring during encapsulation, which can influence the stability and bioactivity of the encapsulated propolis extract.

Microbiological analysis of OECP and MOECP

Table 6 presents the results of the microbiological analysis of the MOECP samples.

The MOECP activity against *Salmonella enteritidis* and *Klebsiella* sp. was null because the bioactive compounds that inhibit these bacterial strains were subjected to spray-drying and volatilization processes or other chemical reactions that altered their functional groups. In addition, the substances in the extract that do not undergo changes during encapsulation lack sufficient activity. This phenomenon was also evidenced -by MOECP activities against gram-positive and coliform organisms such as *Escherichia coli* and microorganisms of the *Enterobacter* genus, whose minimum inhibitory concentrations were higher than in OECP. In this study, the activity against gram-positive microorganisms such as *Staphylococcus aureus* was higher than that of other microparticles obtained from other propolis derivatives, such as ethanolic extracts (Da Cruz Almeida et al. 2017).

Previous studies have investigated microencapsulated with maltodextrin as the wall material, where the encapsulated bioactive compounds were plant extracts such as guava (*Psidium guajava* L) (Fernandes R et al. 2014), cinnamon (*Cinnamomum zeylanicum*) (Ostroschi et al. 2018), soybean (*Glycine max*) (Kalušević et al. 2017) and walnut (*Juglans regia*) (Cheraghali et al. 2018). The results of these studies indicated that none of the bioactive compounds exhibited activity against coliforms or gram-positive bacteria. Therefore, the results presented in Table 6 mean that MOECP had better *in vitro* antimicrobial

Table 6. Minimum inhibitory concentration (MIC) of propolis OECP- according to various parameters used in the Spray Dryer.

Microorganism (mg mL^{-1})	CMI (mg mL^{-1})								
	Sample (MOECP)								
	1	2	3	4	5	6	7	8	OECP
E. coli	5±0.2^a	5±0.2^a	5±0.2^a	5±0.2^a	5±0.2^a	10±0. 6^b	10±0. 6^b	10±0.6^b	5±0.2^a
Staphylococcus aureus	0.6±0.05^a	1.25±0.10^a	2.5±0.10^b	2.5±0.10^b	2.5±0.10^b	2.5±0.10^b	2.5±0.10^b	5±0.10^c	0.306±0.05^a
Salmonella enteriditis	-	-	-	-	-	-	-	-	5±0.2^a
Enterobacter aerogenes	5±0.10^a	5±0.10^a	5±0.10^a	5±0.10^a	10±0.30^b	10±0.30^b	-	-	5±0.5^a
Enterobacter agglomerans	5±0.20^a	5±0.30^a	10±0.30^b	10±0.30^b	10±0.30^b	10±0.30^b	-	-	10±0.5^a
Klebsiella sp.	-	-	-	-	-	-	-	-	10±0.5^a

Different letters in the same column indicate significant differences (*P*<0.05). MIC: Minimum Inhibitory Concentration. MOECP: Microencapsulated oily extract of Colombian propolis. OECP: Colombian propolis oily extract. -: No antimicrobial activity was evidenced.

activity than other plant extracts because of the high content of bioactives with a bactericidal capacity.

Figure 2 presents the results of the multivariate analysis performed on MOECP for antimicrobial activity.

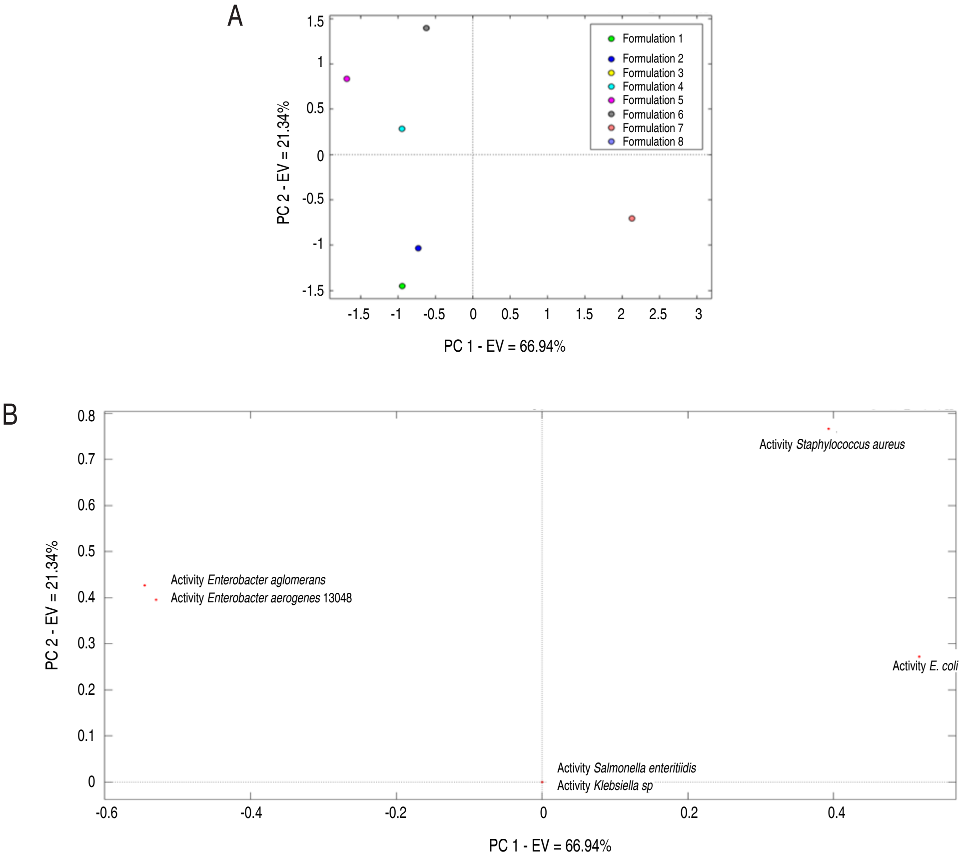

Figure 2. Principal Component Analysis. A) Score plot of microencapsulated propolis oily extracts and B) Loading plots of variables evaluated in the analysis of propolis oily extracts for antimicrobial activity.

One of the most important factors was a clear differentiation of samples according to their biological activity. Only formulations 1 and 2 shared similarities in terms of their bioactivity. Additionally, MOECP activity against *Staphylococcus aureus* and *Escherichia coli* differentiated the samples analyzed better. The activity against microorganisms of the genera *Enterobacter, Klebsiella* sp., and *Salmonella* was similar among the eight formulations, indicating that the drying conditions did not affect the activity against these pathogens. There were only two variables that effectively differentiated the samples, and the multivariate protocol allowed the samples to be differentiated.

Microscopy of the MOECP

Figure 3 shows the morphological characterization of MOECP samples using SEM. It is possible to identify that the microparticles from the oily propolis extract and the mixture of maltodextrin and Gum arabic were mostly spherical although there were amorphous particles with smaller particles were grouped. These clusters were larger in samples with higher concentrations of the oily propolis extract, such as formulations 1 and 2. Lower concentrations and higher temperatures resulted in larger particles with larger contact surfaces and lower densities of particles, possibly because of the higher evaporation rate in these treatments. The particles should be as smooth as possible to facilitates the retention of material. The results showed that temperature was the variable that most affected the micro-encapsulation process and that the samples subjected to higher temperature values had a rougher surface as determined by the microscopy analysis, meaning these particles presented higher bioactive losses.

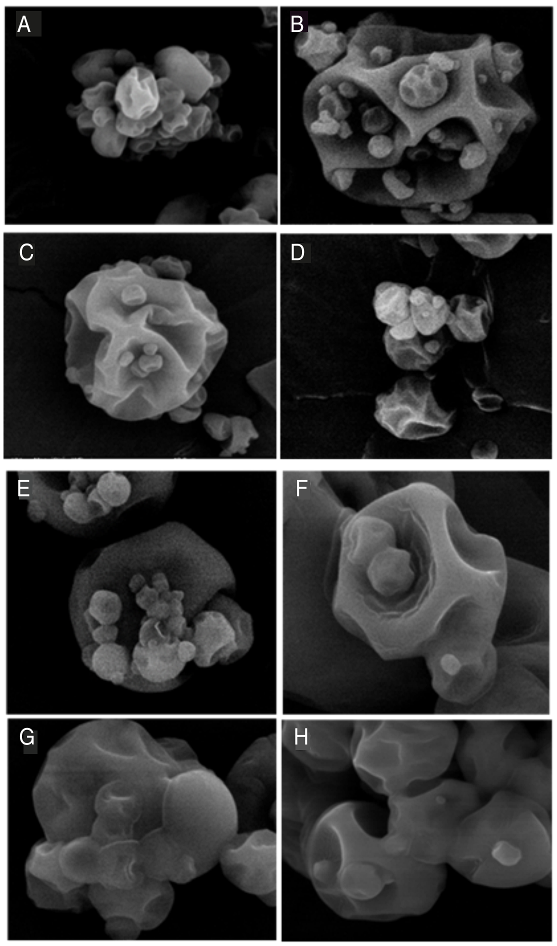

Figure 3. MOECP microparticles at 10 µm magnification. A) Sample 1 (100 °C), B) Sample 2 (120 °C), C) Sample 3 (140 °C), D) Sample 4 (160 °C), E) Sample 5 (100 °C), F) Sample 6 (120 °C), G) Sample 7 (140 °C), H) Sample 8 (160 °C). MOECP: Microencapsulated oily extract of Colombian propolis.

The results of three-dimensional microscopy of MOECP particles are related to their antioxidant activity. It has been reported that, as temperature increases, the presence of defects and cracks increases, which reduces the effectiveness of the wall material because they can degrade through oxidation and hydrolysis processes (Andrade et al. 2017). In terms of morphology, the macroparticles, regardless of their temperature and concentration, retained the same shape, as reported by Ramakrishnan et al. (2018), who used the same wall materials to encapsulate tamarind juice. Maltodextrin was responsible for circular morphology because the proportion of this polysaccharide with respect to Gum arabic was constant. Changes in the shape in the encapsulates were minimal. Microscopy revealed that in the particles with a higher concentration of the extract had more clusters because the spray drying process had a greater mass, which led to microparticles with greater thickness, higher molecular weight, and possibly more clusters (Insang et al. 2022). Samples with more clusters and greater thickness are expected to have a greater extract and, therefore, greater action against pro-oxidant substances and pathogenic bacteria.

DSC analysis of MOECP

Figure 4 and Table 7 show the results of the DSC analysis, which indicate the endothermic and exothermic changes in the MOECP.

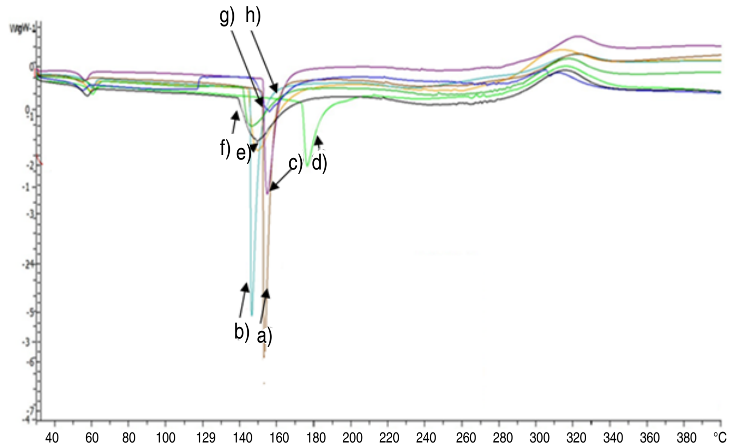

Figure 4. DSC spectra of the MOECPs. a) Light Blue: Sample 1 (100 °C), b) Brown: Sample 2 (120 °C), c) Purple: Sample 3 (140 °C), d) Light Green: Sample 4 (160 °C), e) Yellow Ochre: Sample 5 (100 °C), f) Black: Sample 6 (120 °C), g) Dark Green: Sample 7 (140 °C), h) Dark Blue: Sample 8 (160 °C). MOECP: Microencapsulated oily extract of Colombian propolis.

Table 7. Endothermic and exothermic changes in MOECP based on DSC thermal analysis.

Sample	Endothermic peak heat1 (J g^{-1})	Peak endothermic heat 2 (J g^{-1})	Peak exothermic peak heat (J g^{-1})
1	6.2±0.10[a]	74.7±8.07[a]	100.6±9,34[a]
2	7.7±0.45[a]	119.7±14.19[b]	96±7.20[a]
3	6.3±0.39[a]	118.3±21.05[b]	21.4±3.07[b]
4	4.3±0.31[b]	138.3±17.07[b]	34±4.16[b]
5	6.1±0.54[a]	95.3±8.98[b]	29.8±3.91[b]
6	7±0.62[a]	87.7±8.73[a]	67.8±5.93[c]
7	1.7±0.10[c]	70.7±8.18[a]	60.9±8.04[c]
8	5.4±0.2[b]	63±8.14[b]	7.1±1.01[d]

Samples 1 - 8 = MOECP: Microencapsulated oily extract of Colombian propolis. Different letters in the same column indicate significant differences (P<0.05).

The thermograms showed two endothermic events between 50 and 180 °C, while one exothermic event can be observed between 290 and 320 °C. The samples with a higher concentration of propolis (treatments four to eight) had endothermic peaks with increased height and thickness, while those with a lower concentration of propolis had narrower and weaker peaks. The two melting peaks correspond to Gum arabic and maltodextrin; they presented a difference in intensities, where the peak for Gum arabic (located between 50 and 65 °C) was fainter than that of maltodextrin (located between 150 and 180 °C). In turn, the samples with a higher concentration of the oily propolis extract (1, 2, 3, and 4) had less degradation than those with an extract: encapsulant ratio of (1:3) (5, 6, 7 and 8). It was concluded that, at a higher temperature and lower concentration, the intensity of the endothermic peaks increased, and, consequently, the amount of encapsulated extract decreased. It is recommended to maintain the encapsulation process conditions proposed in formulation two since it had the highest stability as represented by the higher endothermic peak.

The endothermic peak at 50 °C signaled volatilization of water, while the peaks at 150 °C corresponded to fusion processes where there were fusions between low molecular weight compounds, such as volatiles and polyphenols, and the wall material. The exothermic peak between 290 - 320 °C for all extracts indicated the loss of compound and rupture of some microencapsulated particles. This peak was more intense for samples with a higher oily extract concentration because more bioactive compound molecules underwent chemical changes, such as depolymerization and decomposition processes. The higher the intensity of the peaks, the greater the stability of the samples were, therefore, the formulations with higher OECP concentration were more stable. The heat values were higher for formulations 1 and 2, which had a higher concentration of OECP and a lower drying temperature, indicating that the samples were more resistant to high temperatures, pathogens, mechanical forces, degenerative reactions, and other decomposition processes (Ballesteros et al. 2017).

In the future, the addition of a protein system to the elaboration of microparticles, such as ovalbumin and pea protein, can be considered because encapsulates made with various bioactives from these biopolymers have presented more intense endothermic peaks, and exothermic events were not observed that signal the release of secondary metabolites from the particles and, therefore, the restoration of the structural polysaccharides to their original state (Jansen-Alves et al. 2018; Jansen-Alves et al. 2024).

CONCLUSION

OECP was encapsulated using a combination of maltodextrin and Gum arabic. The best microencapsulation seen with the 1:2 encapsulant/extract ratio and a temperature of 120 °C (formulation 2). Physicochemical characteristics and antimicrobial activity were affected by temperature and concentration. At a higher OECP concentration and a lower temperature, better antioxidant activity and greater potential against *Escherichia. coli, Staphylococcus aureus,* and microorganisms of the Enterobacter genera were observed. Formulations with higher concentration and lower drying temperature showed activity against coliforms, which was much higher than that of other encapsulates, where activity against *E. coli* provided a more effective differentiation. The Spray Drying process is effective for protecting the propolis extracts and it can maintain and enhance the activity of raw propolis samples, however, the spraying process volatilizes and degrades labile substances in the extract.

The microencapsulation of OECP enhances or preserves the activity of a sample against pro-oxidant substances and pathogens. Additionally, it allows the stable transport of active ingredients, such as gallic acid and beta-carotene, in a more reproducible manner. These results are important because MOECP could be preserved for much longer than in systems in which propolis was added without encapsulation. Furthermore, depending on the wall material and the optimal microencapsulation temperature/concentration ratio, the effective shelf-life of a bioactive can be extended. Microencapsulation of OECP using mixtures of maltodextrin and Gum arabic is possible, and more than 50% of the phenols and carotenoids in the raw sample are preserved.

A suggestion for future studies related to the encapsulation and preservation of propolis is to incorporate to the wall material formulation, new coatings that have a greater synergism with the terpenoids and phenolic compounds contained in the raw propolis extract that do not suffer denaturation and other chemical reactions that have a negative impact on the bioactivity against pro-oxidant compounds and pathogenic bacteria. The use of coating materials different from polysaccharides such as ovalbumin is strongly recommended due to its synergistic actions with stable high molecular weight terpenoids, carotenoids and phenolic compounds. In addition, new works aimed at studying the encapsulation of propolis should employ other machines with higher speed and efficiency but with the same conditions as those advised in this study. The purpose of this study was to optimize the coating process and to obtain particles with similar conditions but with a higher yield.

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Functional characteristics and antimicrobial activity of supercritical CO_2 extracts from passion fruit (*Passiflora edulis*) seeds

Características funcionales y actividad antimicrobiana de extractos de CO_2 supercrítico de semillas de maracuyá (*Passiflora edulis*)

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ABSTRACT

Keywords:
Antioxidant capacity
By-product
Fatty acid profile
Functional quality indices
RapidOxy

The passion fruit seed is an important byproduct of the agroindustry, as it accounts for 15% of this fruit, so it is imperative to add value to these seeds. In this context, the present study aimed to evaluate the functional characteristics and antimicrobial activity of passion fruit seeds. Oil extraction was carried out using supercritical CO_2 (SC-CO_2) and supercritical CO_2 with ethanol cosolvent (SC-CO_2+et); both oils showed a similar fatty acid profile with a high content of polyunsaturated fatty acids (71.62 and 71.80%, respectively). The study of the functional quality of the oils showed low atherogenicity (AI) and thrombogenicity (TI) indices. The seed flour oil and the defatted passion fruit seed flour extract presented antimicrobial activity against *Klebsiella oxytoca*, *Staphylococcus aureus* and *Proteus vulgaris*. The ethanolic extract of flour defatted with CO_2+ethanol (FDCE) obtained higher values of total phenolic compounds and antioxidant capacity by ABTS and FRAP. The present research provides the characterization of the functional properties of the passion fruit seed oils and the defatted seed, being data of interest for future applications of the passion fruit seed.

RESUMEN

Palabras clave:
Capacidad antioxidante
Subproducto
Perfil de ácidos grasos
Índices de calidad funcional
RapidOxy

La semilla de maracuyá es un residuo agroindustrial importante, ya que representa el 15% de esta fruta, por lo que es imperativo añadir valor a estas semillas. En este contexto, el objetivo del presente estudio fue evaluar las características funcionales y actividad antimicrobiana de la semilla de maracuyá. La extracción de aceite se llevó a cabo utilizando CO_2 supercrítico (SC-CO_2) y CO_2 supercrítico con cosolvente etanol (SC-CO_2+et), ambos aceites mostraron un perfil de ácidos grasos similar con elevado contenido de ácidos grasos poliinsaturados (71,62 y 71,80%, respectivamente). El estudio de la calidad funcional de los aceites presentó índices de aterogenicidad (IA), trombogenicidad (IT) bajos. El aceite de la harina de semilla y el extracto de harina de semilla de maracuyá desgrasada presentaron actividad antimicrobiana frente a *Klebsiella oxytoca, Staphylococcus aureus* y *Proteus vulgaris*. El extracto etanólico de harina desgrasada con CO_2+etanol obtuvo mayores valores de compuestos fenólicos totales y capacidad antioxidante por ABTS y FRAP. La presente investigación proporciona la caracterización de las propiedades funcionales tanto de los aceites de la semilla de maracuyá como la semilla desgrasada, siendo datos de interés para futuras aplicaciones de la semilla de maracuyá.

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The Passifloraceae family has 18 genera and around 630 species distributed in tropical and subtropical areas worldwide; in America, most species are found in Central and South America (Deginani 2001). It is estimated that the agro-industrial waste from the passion fruit juice industry reaches 40% of the amount of processed fruit, and around 90% of the waste is composed of peels and seeds (Malacrida and Jorge 2012). In this context, it is important to use these wastes and study their bioactive compounds to offer alternative uses for these byproducts.

Studies on *Passiflora edulis* show its use as an anti-inflammatory, antimicrobial, lipid-lowering, antioxidant, anxiolytic and antitumor; various types of preparations, extracts and individual compounds derived from this species possess a wide spectrum of pharmacological effects on various organs, as well as on different biochemical processes and physiological functions (Taïwe and Kuete 2017).

Barrales et al. (2015) evaluated the combined effect of seed oil extraction with ultrasound and SC-CO_2, managing to increase overall yield. In relation to the use of passion fruit seed oil, Arturo-Perdomo et al. (2021) and Pantoja-Chamorro et al. (2017) studied the physicochemical composition of passion fruit seed oils obtained by SC-CO_2, highlighting its linoleic acid (67.53%) and sterol content. Santos et al. (2021) and Malacrida and Jorge (2012) reported a potential source of polyunsaturated fatty acids such as linoleic acid in passion fruit seed oil, as well as a source of polyphenols and tocopherol. Dos Santos et al. (2021) evaluated passion fruit oil obtained with supercritical CO_2, reporting high values of linoleic acid in the fatty acid profile, as well as tocotrienol, squalene, and carotenoids. Pereira et al. (2018) evaluated the effect of extraction methods (subcritical propane, Soxhlet and ultrasound) on the composition of passion fruit seed oil, reaching a maximum yield of 26.12%; and also reported antibacterial activity against *Escherichia coli, Salmonella enteritidis, Staphylococcus aureus* and *Bacillus cereus*.

Considering the information presented in studies related to passion fruit seed oil, these have been focused on evaluating the phytochemical profile, extraction methods and their effect on yield; however, no studies were found on functional quality, antioxidant capacity and antimicrobial properties of the oils obtained with two extraction techniques with CO_2 and CO_2+et. In this sense, the present study provides information on the functional characteristics and antimicrobial activity of passion fruit seeds.

MATERIALS AND METHODS

Sample preparation and reagents

The passion fruit (*Passiflora edulis*) seeds, a byproduct of juice production in Pucallpa (Ucayali, Peru), were frozen and transported to the Technological Institute of Production (Callao, Peru). Upon arrival, they were thawed, dried in an oven (Venticell, Ecocell, Switzerland) at 50 °C for 22 hours until reaching 4% moisture, then placed in vacuum bags and stored at -20 °C. For oil extraction, the seeds were ground in an analytical mill (A 11 Basic, IKA, USA), resulting in passion fruit seed flour (PF).

The following reagents were used in this study: Ethanol 99.5% (Scharlau, Spain), hexane ACS (Fermont, Mexico), Fatty Acid Methyl Ester Mix C_4-C_{24} standard mix 37 FAME (Supelco, Germany), methanol HPLC grade (Merck, Germany), Folin 2N (Sigma-Aldrich, Germany), sodium carbonate ACS (Supelco, Canada), Gallic acid monohydrate $\geq$98.5% (Sigma-Aldrich, United States), ABTS (2 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) diammonium salt >=98% (Bio Basic, Canada), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) $\geq$96% (Sigma Aldrich, China), acetic acid $\geq$99.7% (Merck, Germany), TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) 98% (Alfa Aesar, England), Iron (III) chloride hexahydrate ACS (Merck, Germany), carbon dioxide 99.5% v/v liquefied gas (Linde, Peru), nitrogen atmosphere Ultrapure (Linde, Peru), oxygen Ultrapure (Praxair, Peru). For the microbiological assays, all strains were obtained in Kwik Stik format (Microbiologics, United States). The following materials were also used: McFarland standard tube No. 0.5 (Liofilchem, Italy), Mueller-Hinton agar (Condalab, Spain), dimethyl sulfoxide (BioBasic, Canada), and Penicillin disc (OXOID, United Kingdom).

Passion fruit seed oil extraction

Supercritical CO_2 (SC-CO_2)

The extraction of PF oil with SC-CO_2 was carried out in duplicate using the multisolvent equipment (Top industries, 2802 0000) described by Barriga-Sánchez et al. (2022), a cell reducer was used in the reactor (87 cm^3, dimensions: 2.8 cm internal diameter and 14.1 cm inner height). A

40.88±3.39 g of PF was used, and the process parameters were 220 bar, 50 °C, CO_2 flow of 40 g min^{-1} and time of 240 min. The resulting flour will be referred to as CO_2-defatted flour (PFDC), which was stored at -20 °C for subsequent analysis.

Supercritical CO_2 and ethanol cosolvent

A 50.23±0.5 g of PF was weighed in the extraction cell of the multi-solvent equipment, and the extraction was carried out as described by Barriga-Sánchez et al. (2022). The pressure, temperature and CO_2 flow were 220 bar, 50 °C and CO_2 flow of 40 g min^{-1}, respectively. The sample: ethanol ratio was 1: 16 and extraction time was 180 min. The ethanolic extract was concentrated in a rotary evaporator (Buchi, R-300, Switzerland) until dry, then nitrogen was added to eliminate traces of the solvent. Oil extraction was performed in duplicate. The flour defatted with CO_2+ethanol (PFDCE) was stored at -20 °C for subsequent analysis.

Solid-liquid extraction by Soxhlet apparatus

The extraction was carried out using the Soxhlet apparatus (FatExtractor E-500, Buchi, Switzerland). Three grams of PF was weighed and hexane was used as a solvent, the extraction time was 3 h and two replicates were carried out.

Extraction of bioactive compounds from defatted passion fruit flour

Extraction of bioactive compounds from PFDC and PFDCE was performed according to the recommendations of Reis et al. (2020). 70% ethanol was used in a 1: 5 ratio (flour: solvent, w: v) in a thermostatic bath (MEMMERT, WNB 7 - 45, Germany) at 45 °C for 1 h. Subsequently, it was placed on a rotary shaker at 70 RPM for 2 h (MX-RL-Pro, Dragon Lab, USA), centrifuged (Centrifuge 5804 R, Eppendorf, Brazil) at 4 °C for 10 min at 3,200 g, and the ethanolic phase containing the bioactive compounds was recovered and stored at -20 °C for subsequent analysis.

Extraction yield and oil recovery

The extraction oil yield, expressed as a percentage, was determined using Equation 1.

$$\text{Extraction oil yield}(\%) = \frac{W_1}{W_2} \text{x } 100\% \qquad (1)$$

Where: W_1 is the mass of the oil (g) obtained after extraction and W_2 is the mass of the passion fruit seed (g). The oil recovery, expressed as a percentage (%), was calculated using Equation 2.

$$\text{Oil recovery}(\%) = \frac{R_1}{R_2} \text{x}100\% \qquad (2)$$

Where: R_1 is the extraction oil yield obtained by supercritical extraction and R_2 is the extraction oil yield obtained by Soxhlet using hexane.

Fatty acid profile

The methodology described by Barriga-Sánchez et al. (2021) was followed. A chromatograph with an FID detector (Autosystem XL, Perkin Elmer, USA) was used; the oil sample was saponified and methylated prior to analysis. The hot methylation process was carried out using sodium methoxide, followed by acidification with sulfuric acid in methanol and subsequent heating. Fatty acid peaks were identified by comparison with the retention times of the mixture of C4-C24 fatty acid methyl esters. Peak area was calculated using TotalChrom Navigator software and the percentage of each fatty acid was calculated by comparing the individual area of each peak to the total fatty acid area. The analysis for each oil were performed in duplicate.

Functional oil quality

The functional quality of the oil was determined with the data of the fatty acid profile, which were the atherogenicity index (AI) according to Equation 3 (Ratusz et al. 2018), the thrombogenicity index (TI) according to Equation 4 (Ratusz et al. 2018) and the hypocholesterolemic/hypercholesterolemic ratio (H/h) according to Equation 5 (Santos-Silva et al. 2002).

$$AI = \frac{(C12:0 + 4(C14:0) + (C16:0)}{(\sum MUFA) + (\sum \omega - 6) + (\sum \omega - 3)} \qquad (3)$$

$$TI = \frac{(C14:0) + (C16:0) + (C18:0)}{0.5(\sum MUFA) + 0.5(\sum \omega - 6) + (\sum \omega - 3) + (\frac{\sum \omega - 3}{\sum \omega - 6})} \qquad (4)$$

$$\frac{H}{h} = \frac{(C18:1\omega-9)+(C18:2\omega-6)+(C20:4\omega-6)+(C18:3\omega-3)+(C20:5\omega-3)+(C22:5\omega-3)+(C22:6\omega-3)}{(C14:0)+(C16:0)} \quad (5)$$

Where: C12:0 (lauric acid); C14:0 (myristic acid); C16:0 (palmitic acid); C18:0 (stearic acid); C18:1 ω-9 (oleic acid); C18:2 ω-6 (linoleic acid); C18:3 ω-3 (linolenic acid); C20:4 ω-6 (arachidonic acid); C20:5 ω-3 EPA (eicosapentaenoic acid); C22:5 ω-3 DPA (docosapentaenoic acid); C22:6 ω-3 DHA (docosahexaenoic acid); MUFA (Monounsaturated Fatty Acids).

Induction period

The induction period of the oils was measured in a RapidOxy reactor (Anton Paar, Blankenfelde-Mahlow, Germany), 4 g of sample was weighted onto the plate, which was placed in the equipment, the safety cover closed automatically, and the process started. The parameters used were a temperature of 140 °C and a pressure of 700 kPa (Rodríguez et al. 2021). The induction time was calculated with the OXISoft™ software when a 10% decrease in O_2 pressure was reached according to the equipment indication. Each oil was analyzed in duplicate.

Total phenolic compounds (TPC)

To determine the total polyphenol content in oil, the methodology described by Varas et al. (2020) was used, 0.5 g of oil was weighed in a test tube, then 1.5 mL of 90% methanol was added, vortexed for 4 min, then centrifuged at 3,000 rpm for 5 min, then the supernatant was recovered. The process was carried out twice more and then the extract was evaporated to dryness.

Ethanolic extracts PFDC and PFDCE were used to quantify the TPC as described by Barriga-Sánchez et al. (2021) by performing a 5-point gallic acid calibration curve between 50 to 400 ppm (y = 2.18X + 0.02, R^2 = 0.9980), the Folin reagent was added and allowed to rest for 8 min, then 6% sodium carbonate and water were added, leaving it to rest for 1 h for its reading at 750 nm. Analyzes were performed in triplicate and expressed as mg gallic acid equivalent (GAE) 100 g^{-1} sample.

Antioxidant capacity

The antioxidant capacity of the oils obtained by SC-CO_2 and SC-CO_2+et, and the ethanolic extracts PFDC and PFDCE were determined in duplicate and by two methods:

ABTS

Measurement of ABTS (2-2′-Azino-bis(3-ethyl benzothiazoline-6-sulfonic acid)) radical cation scavenging capacity was performed according to Prior et al. (2005). For the test, the ABTS radical cationic solution was prepared in ethanol, reaching the absorbance of 0.70±0.02 at 734 nm. The absorbance of the mixture was measured in a spectrophotometer (Genesys 180, Thermo Scientific, USA) after 30 min, using ethanol as a blank. The reference curve was constructed with 5 concentrations between 0.1 to 2.0 mM of Trolox (Sigma-Aldrich, China) in ethanol (y = 0.31X + 0.01, R^2 = 0.9975). The results were expressed as µmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent (TE) g^{-1} oil and µmol TE g^{-1} extract (db).

FRAP

The methodology of Benzie and Strain (1996) was followed. The FRAP (Ferric Reducing Antioxidant Power) reagent was prepared by mixing acetate buffer solution: TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution: Fe solution in the ratio 25: 2.5: 2.5. Trolox was used as a standard for 5 points of the calibration curve between concentrations of 50 to 600 µM (y = 1.04x + 0.16, R^2 = 0.9980), adding distilled water and FRAP reagent, letting it rest for 30 min, reading at 595 nm. Before reading, the sample was filtered with the 0.2 µm PTFE syringe filter. The results were expressed as µmol TE g^{-1} oil and µmol TE g^{-1} extract (db).

Evaluation of antimicrobial activity

The antimicrobial activity of oils and PFDC and PFDCE dry extracts was carried out with the disk diffusion technique in Mueller Hinton agar according to the methodologies of Gómez et al. (2015) and Cecchini et al. (2018).

The strains studied (*E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Klebsiella oxytoca* ATCC 700324, *Enterococcus faecalis* ATCC 29212, *Salmonella enterica* subsp. *enterica serovar typhimurium* ATCC 14028, *Staphylococcus aureus* subsp. *Aureus* ATTC 25923, *Staphylococos epidermidis* ATCC 12228, *Proteus vulgaris* ATCC 8427, *Shiguella flexeri* ATCC 12022) were incubated in brain heart infusion for 18 h at 37 °C; then these cultures were inoculated in 5 mL of 0.85% w/v saline solution. The concentrations were adjusted with the McFarland standard tube No. 0.5 (1.5 x 10^8 CFU mL^{-1}). Subsequently, 100 µL of each culture was inoculated in Petri dishes with Mueller-Hinton agar, distributing it evenly with a Drigalsky spatula. In each plate, 3 wells of 6 mm diameter were made by perforating the agar with a sterile punch. 50 µL of each sample dissolved in dimethyl sulfoxide (DMSO) at a concentration of 30 mg of dry extract mL^{-1} was placed in each well. DMSO was used as a negative control and Penicillin disc (10 µg) was used as a positive control. The plates were incubated at 37 °C for 24 h. Tests were performed in triplicate for each strain.

RESULTS AND DISCUSSION

Extraction yield and oil recovery of PF

The overall oil yield was 21.75±0.22% and 22.80±0.15%, with the SC-CO_2 and SC-CO_2+et techniques, respectively, with a significant difference (P<0.05) between both extraction techniques. The difference in yields can be attributed to the different solubilities of the solvents. The solubility of SC-CO_2 is comparable to that of organic solvents, which significantly enhances its solvation capacity; this phenomenon is due to the variations in the density of the fluid during the extraction of non-polar compounds (Abbas et al. 2008). While, when using the SC-CO_2+et technique, CO_2 is used and adding ethanol as a cosolvent increases the solubility of the compounds, resulting in greater efficiency in the extraction process, allowing not only the obtaining of non-polar products but also of polar compounds; this is attributed to the hydrogen bonding interactions between ethanol and polar solutes, which significantly improves the extraction yield (Chai et al. 2020). Furthermore, ethanol has been shown to be the most effective solvent for the extraction of polar compounds in supercritical CO_2 systems (Asep et al. 2013). Pantoja-Chamorro et al. (2017) found a yield of 22.23% in dry passion fruit seed after 450 min of extraction with SC-CO_2 (275 bar and 50 °C), close to what was obtained in the present study for 240 min. Dos Santos et al. (2021) reported lower overall yield contents between 14.36 to 17.22% in passion fruit seed oil with SC-CO_2. The lower yield could be due to the origin of the sample studied (Dos Santos et al. 2021), among other factors.

The PF oil recovery was 90.98±0.93% and 95.37±0.63% with the SC-CO_2 and SC-CO_2+et techniques, respectively, based on the overall oil yield obtained with hexane (23.91±0.05%). Other authors also obtained recovery percentages lower than 100%. Antoniassi et al. (2022) reported a 28% yield of PF oil and lower recovery values of passion fruit seed oil (81.4 to 89.0%) obtained from different companies that process passion fruit juice in Brazil. Likewise, Reis et al. (2023) performed the extraction of 15% oil from *Passiflora cincinnata* seeds by pressing using petroleum ether and showed a lower recovery (79%) than in this study. On the other hand, Pereira et al. (2017) reported a higher overall yield in Brazilian passion fruit seeds obtained with hexane (28.33% db). This difference could be due to the usage of enzymes for complete removal in the separation of seeds from passion fruit pulp. In this study, an industrial byproduct of seeds was used after the complete extraction of the juice, so there was the presence of passion fruit pulp which could reduce the overall yield of oil extraction since the pulp does not contain oil in its composition.

Fatty acid profile

The results show 86.58 and 86.51% unsaturated fatty acids, and 13.42 and 13.49% saturated fatty acids in oils obtained with SC-CO_2 and SC-CO_2+et, respectively (Table 1), which are higher in unsaturated and lower in saturated fatty acids compared to the values reported by Pantoja-Chamorro et al. (2017) for passion fruit seeds from Colombia (84% unsaturated and 15.45% saturated fatty acids). Reis et al. (2023) reported a higher palmitic (12.14±1.00%) and linoleic (78.34±2.22%), and lower stearic (1.09±0.26%) and oleic (8.43±1.32%) contents in *Passiflora cincinnata* seed oil (extracted in a continuous press) compared to this study. The difference in fatty acid contents could be attributed to the passion fruit variety and its growing area.

Table 1. Fatty acid content in (%), functional quality indices and induction period.

Component	$SC\text{-}CO_2$	$SC\text{-}CO_2$+et
C 14:0 (Myristic)	0.06±0.01[a]	0.06±0.0[a]
C 16:0 (Palmitic)	10.23±0.01[a]	10.36±0.06[a]
C 16:1 (Palmitoleic)	0.14±0.0[a]	0.14±0.01[a]
C 17:0 (Heptadecaenoic)	0.07±0.01[a]	0.07±0.0[a]
C 18:0 (Stearic)	2.82±0.01[a]	2.78±0.03[a]
C 18:1 w-9 (Oleic)	13.99±0.01[a]	13.8±0.21[a]
C 18:1 w-7 (Vaccenico)	0.58±0.01[a]	0.52±0.07[a]
C 18:2 w-6 (Linoleic)	71.06±0.01[a]	71.27±0.33[a]
C 18:3 w-3 (α-Linolenic)	0.47±0.01[a]	0.46±0.01[a]
C 20:0 (Arachidic)	0.18±0.0[a]	0.17±0.0[b]
C 20:1 w-9 (Eicosaenoic)	0.13±0.01[a]	0.12±0.0[a]
C 24:0 (Lignoceric)	0.06±0.0[a]	0.05±0.01[a]
C 22:5 w-3 (Docosapentaenoic)	0.09±0.01[a]	0.07±0.0[a]
C 24:1 w-9 (Nervonic)	0.12±0.0[b]	0.13±0.0[a]
Saturated	13.42±0.02[a]	13.49±0.08[a]
Monounsaturated	14.96±0.00[a]	14.71±0.28[a]
Polyunsaturated	71.62±0.01[a]	71.80±0.34[a]
Unsaturated/saturated	6.46±0.01[a]	6.41±0.05[a]
AI	0.121±0.00[a]	0.123±0.00[a]
TI	0.293±0.00[a]	0.296±0.00[a]
H/h	8.326±0.00[a]	8.214± 0.00[a]
Induction period (min)	40.53±0.63[a]	89.05±0.42[b]

Values are expressed as the mean ± standard deviation (n=2). Different letters within the same column indicate a significant difference (P<0.05) according to the t-test.

Functional oil quality

Passion fruit seed oils extracted with supercritical fluids showed AI and TI values, close to zero, being considered favorable in the prevention of coronary heart diseases (Pinto et al. 2020). Pham-Huy et al. (2008) state that the fatty acids present in passion fruit oil are essential for the prevention of cardiovascular diseases, the control of hypertension and the strengthening of the immune system. In fact, the consumption of foods with lower AI is associated with a reduction in total and LDL cholesterol levels in human blood plasma; and the consumption of foods with a lower TI is beneficial for cardiovascular health (Chen and Liu 2020).

There are few reports of studies on functional oil quality indices, one of them is from Barriga-Sánchez et al. (2021), who reported AI (0.20) and TI (0.23) values in *Vitis labrusca* grape seed oil; and Santos et al. (2021), who reported AI (0.16) and TI (0.40) values in passion fruit oil extracted by Soxhlet. The observed differences in values compared to the present study can be attributed to the type of extraction method used. Conventional techniques like Soxhlet employ organic solvents, which, while effective, can degrade the oil during solvent removal. This degradation can impact the fatty acid composition and, consequently, the quality indices of the oil. Therefore, it is crucial to use appropriate technologies for oil extraction and avoid its degradation.

On the other hand, low values of the H/h ratio are considered unfavorable and can induce an increase in cholesterolemia (Santos-Silva et al. 2002). The H/h ratio values of passion fruit seed oil obtained with both methods presented lower values than camelina oil (11.2 to 15.0) reported by Ratusz et al. (2018), but higher than in passion fruit seed oil reported by Santos et al. (2021) (H/h=6.03); this difference is due to the extraction technique used. It is important to determine the quality indices since they are related to growth, development, maintenance of various functions of human metabolism and the promotion of good health (Santos et al. 2021).

Induction time

The induction time of the oils was measured to evaluate their oxidative stability and compare the stability of both oil samples. Despite having a similar fatty acid profile, the oil extracted with SC-CO_2+et presented a higher induction time value (Table 1). The shorter induction time of SC-CO_2 oil (40.53 min) suggests it may be more susceptible to oxidation compared to the SC-CO_2+ethanol oil (89.05 min). Consequently, the oil extracted with SC-CO_2+ethanol can be considered more stable, likely due to the presence of polar compounds such as phenols, which contribute antioxidant properties and help extend the oil's shelf life.

Other studies, such as Lau et al. (2006) reported a similar trend in palm-pressed fiber oils where the oil obtained with SC-CO_2+et presented greater oxidative stability measured by Rancimat than the oil extracted with SC-CO_2. Furthermore, Reis et al. (2020) found longer induction times at 110 °C for *Passiflora alata* (3.52 h), *Passiflora setacea* (7.32 h) and *Passiflora tenuifila* (6.87 h). This could be attributed to the differences in the variety studied, as the fatty acid content differs depending on the plant variety. It is known that the double bonds of linoleic acid are 40 times more unstable than those of oleic acid, which has a single bond (Damodaran and Parkin 2017).

TPC and Antioxidant capacity of oil and extracts

The oil extracted with SC-CO_2+et showed higher TPC content and antioxidant capacity, as measured by both the ABTS and FRAP assays (Table 2), compared to the oil obtained using SC-CO_2 in this study. These results were also higher than those reported by Ribeiro et al. (2020), who extracted oil using pressurized ethanol, obtaining 8.54 mg GAE g^{-1} and an ABTS antioxidant capacity of 18.2 µmol TE g^{-1}. Furthermore, it was also higher than the ABTS antioxidant capacity of 0.73 µmol TE g^{-1} obtained through Soxhlet extraction with diethyl ether in Brazilian *Passiflora* seed oils (de Santana et al. 2015). This difference can be attributed to the use of ethanol as a cosolvent in the extraction with supercritical CO_2, which improves the solubility of polyphenols (Rahal et al. 2015). Likewise, when a higher TPC content is present, it allows greater electron donation and greater synergistic antioxidant capacity on ABTS free radicals (Purohit et al. 2021) and the ability of the antioxidants present in the oil to reduce the ferric ion to its ferrous form (Pereira et al. 2017).

In general, fruit seeds, often considered byproducts of juice processing, contain oil rich in bioactive compounds such as tocopherols, carotenoids, flavonoids, phenolic acids, and phytosterols (Kaseke et al. 2020). These compounds are responsible for the antioxidant properties of the oil.

Table 2. TPC and antioxidant capacity of passion fruit seed oil and defatted passion fruit seed extract with CO_2 (PFDC) and CO_2+ethanol (PFDCE).

Sample	TPC (mg GAE 100 g^{-1})	ABTS (µmol TE g^{-1})	FRAP (µmol TE g^{-1})
Oil obtained by SC-CO_2	65.92±1.78^a	16.39±0.02^a	41.41±0.15^a
Oil obtained by SC-CO_2+et	251.78±1.07^b	21.83±0.06^b	63.43±1.54^b
PFDC extract	1049.66±28.92^A	80.47±1.19^A	75.14±4.07^A
PFDCE extract	1243.80±62.45^B	117.79±5.65^B	111.40±3.53^B

Values are expressed as the mean ± standard deviation (n=2). Different lowercase letters within the same column indicate a significant difference (P<0.05) according to the Tukey test. Different capital letters within the same column indicate a significant difference (P<0.05) according to the Tukey test.

PFDCE extract presents higher values of TPC and antioxidant capacity (Table 2) than PFDC extract (P<0.05). Malik et al. (2023) reported a value of 19,360 mg EAG 100 g^{-1} TPC of defatted sample in acetone extract and found improved solubility in contrast to the ethanol reported in this study. Da Costa et al. (2023) evaluated the extract (50% acetone) of purple passion fruit seed and reported 758.43±7.79 mg EAG 100 g^{-1} and 125.207 µmol g^{-1} for TPC and ABTS, respectively. Reis et al. (2023), after optimizing ethanol concentration and solid-liquid ratio for extracting antioxidant compounds from defatted *Passiflora cincinnata* seeds, reported higher values of 2,868 mg GAE 100 g^{-1} and ABTS antioxidant capacity (195 µmol TE g^{-1}) than those found in the present study. The higher ABTS and FRAP values in both SC-CO_2+et oil and PFDCE extract may be attributed to the increased total phenolic content. In a previous study, Reis et al. (2020) also reported lower TPC values in ethanolic extracts from *Passiflora alata* and *Passiflora tenuifila* seeds but higher ABTS values.

Antimicrobial activity

The antimicrobial activity of the passion fruit seed oil and defatted passion fruit seed dry extract samples against gram (+) and gram (-) bacterial strains is shown in Table 3. The results show that the passion fruit seed oil extracted with SC-CO_2 and SC-CO_2+et presented antimicrobial activity against the bacteria *Escherichia coli, Klebsiella oxytoca, Salmonella enterica, Staphylococcus aureus* and *Proteus vulgaris* with larger halo sizes for the oil obtained with SC-CO_2+et, this positive effect would be due to the composition of fatty acids, antioxidants and flavonoids of passion fruit oils (Purohit et al. 2021).

Pereira et al. (2018) evaluated the antibacterial effect of passion fruit seed oil extracted by Soxhlet against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enteritidis*. Their results demonstrated the oil's antimicrobial effect on these bacteria, which is consistent with the findings of the present study. It is important to mention that the oils did not register an effect on the microorganisms *Enterococcus faecalis* and *Staphylococcus epidermidis*, possibly due to the resistance of the cell walls of these bacteria, which prevented the penetration of the oil (Fathi-Achachlouei et al. 2020). PFDC and PFDCE dry extracts showed greater antimicrobial activity against the bacteria *Klebsiella oxytoca, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis* and *Proteus vulgaris*. The greatest halo of inhibition was formed by PFDC dry extract.

Noguera-Machado et al. (2017) evaluated the antibacterial potential of ethanolic extracts of *Passiflora edulis* seeds, showing the greatest antibacterial potential on strains of *S. aureus* and *E. coli*, with a bactericidal effect at concentrations of 11.7 and 9.4 mg mL^{-1}, lower concentrations than those analyzed in this study. PFDC and PFDCE dry extracts at a concentration of 30 mg mL^{-1} showed antimicrobial activity against *Staphylococcus aureus* with a halo size of 17 and 15 mm, respectively, which is consistent with that reported by Nugraha et al. (2018), who evaluated the antibacterial effect of the ethyl fraction of the peel of *Passiflora edulis* against S*taphylococcus aureus*, finding inhibition zones of 14.23, 19.53 and 20.43 mm for concentrations of 100, 400 and 500 mg mL^{-1}, respectively. The antibacterial activity of the defatted passion fruit extracts could be attributed to the effect of phenolic compounds and the effectiveness of the solvent used to recover the highest amount of polyphenols (Ramaiya et al. 2014).

Table 3. Antimicrobial activity in passion fruit seed oils and defatted passion fruit seed extracts.

Bacteria	Halo of inhibition (mm)			
	Oil		Extract*	
	SC-CO_2	SC-CO_2+et	PFDC	PFDCE
Escherichia coli	7	9	0	0
Klebsiella oxytoca	7	10	17	12
Enterococos faecalis	0	0	17	10
Salmonella enterica	9	11	0	0
Staphylococcus aureus	8	10	17	15
Staphylococcus epidermidis	0	0	18	16
Proteus vulgaris	7	9	16	14

PFDC: Defatted passion fruit seed extract with CO_2.
PFDCE: Defatted passion fruit seed extract with CO_2 ethanol.
*: 30 mg of dry extract mL^{-1} DMSO.

CONCLUSION

The present study showed that the fatty acid profile of the oils obtained with SC-CO_2 and SC-CO_2+et did not present a significant difference and that the functional quality indices (AI, TI and H/h) of the passion fruit seed oils obtained by the two techniques are notable. However, the oil obtained with SC-CO_2+et, as well as the ethanolic extract of defatted passion fruit seed, presented a higher content of phenolic compounds and antioxidant activity. Furthermore, the oil extracted using SC-CO_2+et exhibited greater oxidative stability compared to the oil obtained through SC-CO_2 alone. The antimicrobial activity of the oil against pathogens of interest to the food industry was demonstrated, as well as that of the dry extracts obtained from defatted flours. This research provides valuable insights for the food industry, particularly for those seeking to repurpose waste materials into new products with functional properties.

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CONFLICT OF INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Evaluating the Andean blueberry (*Vaccinium meridionale* Swartz) powder in the preparation of ice cream: Improving the antioxidant capacity and the total phenolic content

Evaluación del polvo de arándano Andino (*Vaccinium meridionale* Swartz) en la preparación de helado: Una mejora a la capacidad antioxidante y del contenido de fenoles totales

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ABSTRACT

Keywords:
Agraz powder
Ice cream
Nutraceutical
Polyphenols
Stability

This study evaluated the content of total phenolic compounds, antioxidant capacity, and functional properties of an ice cream formulated from Andean blueberry (*Vaccinium meridionale Swartz*) powder. The antioxidant capacity of pulp and powder from Andean blueberry was performed by using ABTS*+ and DPPH methods. The blueberry powder was obtained by convection drying, and the rheological and thermal properties of the ice cream were determined by rotational rheology and differential scanning calorimetry (DSC), respectively. The stability of ice cream was evaluated at -15 °C during storage for 30 days, measuring color, texture, antioxidant capacity, and phenolic compounds. The results showed a moisture content of 77.64% (w.b), 13.59 °Brix, pH of 3.0, and titratable acidity of 1.35% for the Andean blueberry pulp. The color parameters L*(10.2), a*(3.5), and b*(7.4) indicated the typical dark-purple color of this fruit. Andean blueberry powder shows a moisture content of 5.20% (w.b), solubility of 58.24% in water, and color parameters' values of L*(16.0), a*(16.9) and b*(8.1), with 1.43 mg GAE g^{-1} of total phenol, while, for the pulp, it was 4.31 GAE g^{-1}. The antioxidant capacity ranged between 200 and 161 µmol TEs g^{-1} dry mass for pulp and from 56 to 49 µmol TEs g^{-1} dry mass for powder. Adding powder from Andean blueberry in the ice cream decreases overrun and delays the melting time. Finally, the ice cream showed good stability during storage time, keeping the color, and texture properties such as hardness, total phenolic compounds, and antioxidant capacity.

RESUMEN

Palabras clave:
Polvo de agraz
Helado
Nutracéutico
Polifenoles
Estabilidad

Este estudio evaluó el contenido de compuestos fenólicos totales, la capacidad antioxidante y las propiedades funcionales de un helado formulado a partir de polvo de arándano andino (*Vaccinium meridionale Swartz*) obtenido por secado convectivo. La capacidad antioxidante de la pulpa y del polvo se llevó a cabo utilizando los métodos ABTS*+ y DPPH. Las propiedades reológicas y térmicas del helado se determinaron mediante reología rotacional y calorimetría diferencial de barrido, respectivamente. Se evaluó la estabilidad del helado a -15 °C durante 30 días, se midió el color, la textura, la capacidad antioxidante y los compuestos fenólicos. Los resultados mostraron un contenido de humedad de 77,64% (b.h), 13,59 °Brix, pH de 3,0 y una acidez titulable de 1,35% para la pulpa. Los parámetros de color L*(10,2), a*(3,5), b*(7,4) mostraron el típico color púrpura oscuro de esta fruta. El polvo presentó un contenido de humedad de 5,2% (b.h), solubilidad en agua de 58,24% y los parámetros de color L* de 16,0, a* de 16,9 y b* 8,1, con 1,43 mg GAE g^{-1} de fenoles totales, mientras que en la pulpa fue de 4,31 GAE g^{-1}. La capacidad antioxidante osciló entre 200 y 161 µmol TEs g^{-1} de masa seca para pulpa y entre 56 y 49 µmol TEs g^{-1} de masa seca para polvo. La adición de polvo de arándano andino disminuye el overrun y retrasa el tiempo de derretimiento. El helado mostró buena estabilidad durante el tiempo de almacenamiento, manteniendo el color, la dureza, los compuestos fenólicos totales y su capacidad antioxidante.

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Ice cream is a sweet-tasting product consumed frozen, with a creamy consistency due to air incorporated in its structure. In addition to water and sugar, it contains dairy components, aromatic substances, colorants, thickeners, stabilizers, and emulsifiers. Allied Market Research (2018) estimates that the global ice cream market will reach US $ 97.301 million by 2023, with an annual growth rate of 5.4% from 2017 to 2023. Currently, consumers are more disposed to pay for plant-based ice cream prepared from natural ingredients, which offer more benefits than traditional or conventional ice cream (Sloan 2019). This aspect has increased the interest of researchers in developing products that combine health and nutrition, incorporating the nutraceutical properties of some natural ingredients. For example, Ginger (*Zingiber officinale*) has been added to ice cream formulation, leading to the obtaining of a product with less total lipids, high antioxidant capacity, and more content of phenolic compounds (Gabbi et al. 2018). The addition of polyphenols may improve cardiovascular functions, and physical performance, and offset oxidative stress in healthy individuals, athletes, the elderly, and in patients suffering from some chronic and degenerative diseases (Sanguigni et al. 2017). Nevertheless, these kind of compounds can also cause significant changes in the rheological and sensory properties of products (Borrin et al. 2018). Çam et al. (2013) evaluated the addition of phenolic compounds extracted from the peel of pomegranate and the oil of its seed. They reported significant changes in the pH, total acidity, and color of the samples. However, the most outstanding results were notable improvements in antioxidant and antidiabetic activities, as well as the increase in phenolic compounds. In another study, Kavaz et al. (2016) used dried Besni grape, reporting changes in the content of phenolic compounds and viscosity, but without changes in the sensory acceptability, taste, or texture. Phenolic compounds are secondary metabolites of the plants, which are classified as flavonoids and non-flavonoids, and are constituted by a structure of phenyl benzopyran with two phenyl rings liked by a heterocyclic pyran ring, which can inhibit the reactive oxygen species (de la Rosa et al. 2019). Andean blueberry (*Vaccinium meridionale Swartz*) commonly known as agraz is a source of phenolic compounds that can be consumed fresh, in liqueurs, jams, or desserts. Physiologically, this plant is a small shrub whose fruits are globose berries of green color in an immature state and dark purple, almost black, in its mature state. It has a diameter that oscillates between 7 and 15 mm (Buitrago et al. 2015). Like other Vaccinium, they have a high content of phenols (8875.3 μg g^{-1} sample) and anthocyanins (5386.4 μg g^{-1} sample), with high antioxidant capacity with value of 278.2 μmol TEs g^{-1} determined from ABTS and 85.1 μmol TEs g^{-1} using DPPH (Moyer et al. 2002). To evaluate the antioxidant capacity, it is recommended to use two methodologies to obtain reliable results. The ABTS method analyzes hydrophilic antioxidants, and the DPPH method analyzes the lipophilic antioxidants present in the Andean blueberry (Baenas et al. 2020).

Phenolic compounds have a wide range of biological functions and antimicrobial activity, they can chelate iron and copper ions, inhibit lipoxygenase, and avoid the proliferation of cancer cells. Besides, their structure allows the stabilization of free radicals by resonance (Bao et al. 2008). Due to the high consumption level and acceptability of the ice cream by consumers of all ages, we are considering that there is a great opportunity to offer additional benefits to the public, with natural additives that allow for improving the functional character of this product, incorporating fruits with high antioxidant capacity to it, among which it can highlight the Andean blueberry (*Vaccinium meridionale Swartz)*. The current study addressed different aspects of the development of an ice cream added with Agraz powder, evaluating its effect on the thermal and rheological properties, techno-functional properties, the total content of phenolic compounds, and antioxidant capacity.

MATERIALS AND METHODS

Reagents Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium carbonate, potassium persulphate, Folin–Ciocalteau and Chlorogenic ABTS^{*+} (2,20-azino-bis (3-ethylbenzothiaoline-6-sulphonic acid)) reagent were purchased from Merck (USA). Gallic acid, 2,20-azino-bis (3-ethylbenzothiazoline-6- sulphonic acid) diammonium salt (ABTS) was purchased from Sigma Aldrich (St. Louis, MO, USA). Methanol, ferric chloride hexahydrate and liquids were supplied by Fisher Scientific (Fair Lawn, NJ, USA).

Characterization of Andean blueberry pulp

Andean blueberry (*Vaccinium meridionale Swartz*) was acquired in the local market (Antioquia-Colombia). The fruits, packaged in 250 g packs, had an average diameter of 10.4 mm and a ripeness state of 4 (reddish-purple coloration). The fruits were washed, disinfected, and stored in a freezer at -18 °C until used. The color parameters of the pulp were determined by using a X-Rite 939 spectrophotometer (X-Rite, Inc, Michigan, USA). The results were expressed according to CIELAB's (L *, a *, b *) system. The pH, water activity (a_w), moisture content, total titratable acidity, total soluble solids (measured in degrees Brix), and maturity index, were determined according to the AOAC (AOAC 1998) methods 981.12, 978.19, 942.15, 932.05, and 934.06, respectively.

Phenolic compounds

The phenolic compounds were determined using the Folin-ciocalteau method (Singleton et al. 1974). The samples (0.5 g) were mixed with a methanol-water (70:30) solution (30 mL). The absorbance was measured at 760 nm in a spectrophotometer UV/VIS (UV-3000, Shanghai, China). Results were expressed as mg gallic acid equivalents per gram of sample (mg GAEs g^{-1}). All analyses were carried out in triplicate.

Antioxidant capacity by ABTS^{*+} and DPPH assay

The antioxidant capacity was determined by radical ABTS^{*+} capture methodology according to Re et al. (1999). The samples (0.5 g) were mixed with a methanol-water (70:30) solution (30 mL). The absorbance was measured at 734 nm in a spectrophotometer UV/VIS (UV-3000, Shanghai, China). The results were expressed as µmol of Trolox equivalent (TEs) per 100 g dry sample (µmol TEs g^{-1} d.b). The analyses were carried out in triplicate. The DPPH method was used (with some modification) to determine the antioxidant capacity according to Cheung et al. (2003). The extract was obtained from 0.5 g of sample in 30 mL aqueous methanol (70:30) and centrifuged at 8,000xg for 15 min. Then, the supernatant was collected in a 50 mL volumetric flask, and the final volume was measured with 60% methanol: water (70:30) solution. In test tubes, protected from light, 50 µL of the extract was added to 950 µL of DPPH radical 0.25 mM and read at 515 nm in a spectrophotometer UV/VIS (UV-3000, Shanghai, China) after 30 min, using methanol as the blank. The results were expressed as µmol of Trolox equivalent (TEs) per 100 g dry sample (µmol TEs g^{-1} d.b). The analyses were carried out in triplicate.

Andean blueberry powder preparation

Andean blueberry pulp was dried with air heated at 55 °C and a speed of 0.5 m s^{-1}, with a layer thickness of 3 mm, for 24 h (İzli 2017). After the drying process, the samples were cooled with liquid nitrogen and subjected to a size reduction process by using a blade mill (IKA, MF 10.2 Impact Grinding, France). Then, samples were sieved using a Ro-Tap Tyler-type sieve shaker (Model RX29) for 15 min. Samples passed through 30-60 mesh sizes were collected and used in the ice cream formulation.

Andean blueberry powder solubility

The sample (0.5 g) was added to a recipient containing 50 mL of distilled water at 25 °C, and stirred in a vortex at 110 rpm for 30 min, before being centrifuged at 3,100×g for 10 min. The supernatant (25 mL) was transferred to a previously weighed porcelain dish and dried to constant weight in an incubator at 105 °C. The dish was weighed, and the solubility was calculated from the difference in weight.

Ice cream preparation

Hard frozen ice cream formulations A (control) and B, enriched with Andean blueberry powder (1.8%), were formulated and subjected to processing according to the protocols defined in the Laboratory of dairy products of Universidad Nacional de Colombia, Medellín Headquarters: Milk (56%), milk cream (22.45%), sucrose (14.5%), milk powder (6.5%), yolk (0.3%) and stabilizer (0.25%), with fat content of 14% and sweetness of 14.5%. Thus, the ice cream mixes were pasteurized at 85 °C for 25 s, rapidly cooled, and kept at 4 °C for 24 h, for maturation. The ice cream was packed in plastic containers of 20 L and stored in a freezer at -15 °C.

Rheology: Viscosity of the ice cream

The viscosity values were obtained from flow curves. The tests were performed at 5 °C, spindle 6, using a viscometer model DV-III (Brookfield, Engineering Laboratories, Stoughton, MA, USA) with a concentric cylinder geometry. Ice creams (15 g) were carefully sampled without disturbing their structure according to Erkaya et al. (2012). The analyses were carried out in duplicate.

Differential Scanning Calorimetry (DSC)

The thermal characterization of the ice cream was conducted by analyzing the thermograms obtained in a TA Instruments DSC with RCS cooling system (New Castle, DE, USA) according to Hwang et al. (2009). The equipment was calibrated with indium before analysis. An empty aluminum pan was used as the reference. For analysis, 5-6 mg of the samples were weighed in aluminum pans. Scanning of all samples was carried out using the same heating (-30 to 30 °C) and cooling (30 to -30 °C) rate of 5 °C min^{-1}.

Texture properties (hardness)

The evaluation of the textural properties of ice cream was carried out according to Aime et al. (2001). The test was performed in a TA.XT2i texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK). The test and posttest speed was 1 mm s^{-1} using a cylindrical probe, with a diameter of 12 mm and a penetration distance of 30 mm.

Techno functional properties

The overrun was calculated by weighing 100 mL of ice cream before and after whipping. The ice cream was gently scooped into a glass cup of 100 mL and leveled by using a rubber spatula. The mean of three weights was used for overrunning, according to Equation (1):

$$\text{overrun (\%)} = \frac{\text{wt. 100 mL of mixture-wt.100 mL of ice cream}}{\text{wt.100 mL of ice cream (mL)}} \times 100 \quad (1)$$

Melting time, melting resistance, and melting rate

Melting time, melting resistance, and melting rates were determined by using the cone method, according to Góral et al. (2018), with some modifications. The ice cream (100 g) at -18 °C was placed on a mesh (# 100) and taken into a controlled temperature chamber at 25 °C and a relative moisture of 75%. The time for obtaining the first drop of melted ice cream was assumed as melting time. The melting rate (mL min^{-1}) was calculated as the volume of ice cream melted every 5 min, for 45 min.

Stability analyses

The ice cream with Andean blueberry powder added was studied at -15 °C. The parameters of color, texture, phenolic compounds, and antioxidant capacity were measured at 0, 15, and 30 days.

Statistical analyses

The experimental data obtained were expressed as mean ± standard error and subjected to an analysis of variance (ANOVA with α=0.05) for a completely random design, using the STATGRAPHICS Centurion Software (Version XVIII). The differences among mean values were determined by using Tukey's multiple range tests (α=0.05).

RESULTS AND DISCUSSION

Andean blueberry pulp shows a moisture content of 77.64%±0.41 (w.b) and water activity of 0.98, total soluble solid of 13.59±0.22 °Brix, pH=3.0±0.1, titratable acidity of 1.35%±0.2, and a maturity index of 10.10. The color parameters L*(10.2), a*(3.5), and b*(7.4) indicated the typical dark-purple color of a fruit with a sensory-acceptable degree of maturity. Regarding the Andean blueberry (*Vaccinium meridionale*) powder, it reached a moisture content of 5.20%±0.02 (w.b), and significant differences in the color parameters compared to fresh fruit with values for L*, a*, b* of 16.0, 16.9 and 8.1, respectively. The mean value of the total phenols in the pulp was 4.31±0.2 mg GAE g^{-1} (d.b). This value was lower than the total phenols reported by Dróżdż et al. (2017) for the *Vaccinium vitis-idaea* L species, which ranged between 4.68 and 6.61 mg GAE g^{-1} dry sample. Zielinska and Michalska (2016) reported a total phenols for *Vaccinium corymbosum* L of 228±0.07 mg GAE g^{-1}. Phenolic compounds are affected by several factors such as the extraction method, variations in different physiological states, and storage conditions (Garzón et al. 2010). In the current study, the Andean blueberry powder had a solubility of 58.24% in water. The drying process significantly decreased (P<0.05) the total phenols, leading to values of 1.43±0.043 mg GAE g^{-1}. Zielinska and Michalska (2016) evaluated the convective air-drying at 90 °C and microwave vacuum drying of the *Vaccinium corymbosum* L. They reported a total polyphenols content of 2.28±0.07 g GAE g^{-1} dry sample and 1.26±0.02 g GAE g^{-1} dry sample for the unprocessed and dried fruit, respectively. López-Vidaña et al. (2017) reported similar results when they evaluated the effect of temperature in the drying process of agraz (*Vaccinium meridionale* Swartz) in terms of antioxidant capacity. Both studies concluded that after the convection air-drying process, only 35 to 45% of the initial total polyphenols present in the fresh fruit were maintained, the variation in these percentages depends on the time and the temperature of exposure, presenting higher retention in those samples subjected to a higher temperature and less processing time. The results of these studies are comparable with those reported in this study. Since the polyphenols are thermolabile compounds, their degradation during the drying process is expected. Nevertheless, the decrease in the phenols may also be related to the activation of oxidative enzymes during the drying process, such as polyphenol oxidase and peroxidase, which leads to the loss of phenolic complexes (Gümüşay et al. 2015).

Antioxidant capacity

The antioxidant capacity obtained by means of the ABTS*+ and the DPPH methods in the Andean blueberry's (*Vaccinium meridionale* Swartz) pulp, reached values of 200.31 and 161.71 μmol TEs g^{-1} dry mass, respectively. The antioxidant capacity for the powder decreased significantly, with values of 56.79 and 49.76 μmol TEs g^{-1} dry mass for the ABTS*+ and DPPH tests, respectively. These values represent a loss of 30% in the antioxidant capacity of the pulp, due to the action of the drying. This percentage was lower than that reported by Reque et al. (2015), who evaluated the effect of the drying process on different species of the *Vaccinium* family. They reported a loss of antioxidant capacity between 46 and 66%, and values significantly higher by DPPH test, ranging from 2938.05 for juice and 4,958 μmol TEs g^{-1} dry mass for dried fruit, while obtained by ABTS*+ were similar to these reported in this study, with values of 236.74 for the juice and 127.29 μmol TEs g^{-1} dry mass for the dry product. The antioxidant capacity of the Andean blueberry is due to compounds such as Hydroxycinnamic acids (5-O-Caffeoylquinic acid), Anthocyanins (Cyanidin-3-O-hexoside I and Cyanidin-3-O-pentoside), and Flavonols (Quercetin-3-O-hexoside I and Quercetin-3-O-pentoside III) (Baenas et al. 2020). The behavior of the antioxidant capacity determined by the ABTS*+ and DPPH methods showed a similar trend to these observed for the content of total phenols, which decreased with the drying time.

Effect of the addition of Andean blueberry powder on the melting time and melting rate

Ice cream rheology showed a pseudo-plastic behavior, with a continuous breakdown of the structure as a result of the lower resistance to flow after the application of stress (Figure 1). The addition of Andean blueberry powder significantly increases ($P<0.05$) the viscosity of the ice cream. The viscosity obtained with 1.8% of Andean blueberry is less than that reported in the preparation of ice cream added with cape gooseberry pulp at 10%, which is about 3,430±267 mPa s (Erkaya et al. 2012). Also, Erkaya et al. (2012) evaluated the influence of the addition of cape gooseberry (*Physalis peruviana* L.) on the chemical and sensory characteristics of ice cream. They reported that the percentage of added fruit had a significant effect on apparent viscosity, with values of 1,714±102 mPa s in the control sample and of 4,654±339 mPa s when 15% of cape gooseberry was added. These values

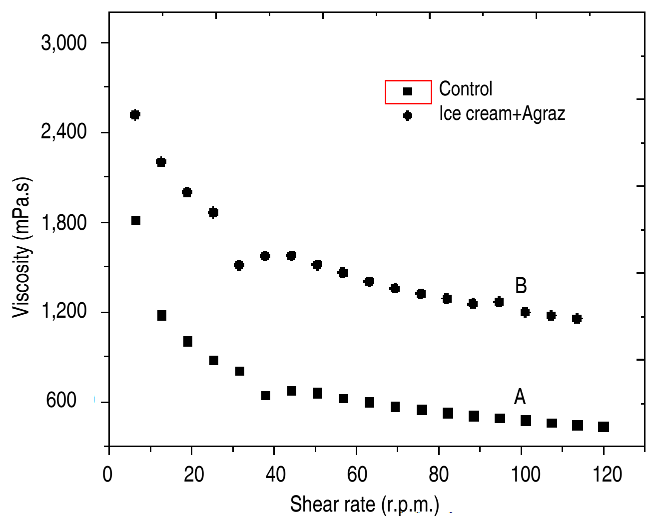

Figure 1. Effect of shear rate on the apparent viscosity of ice creams. **A**. control and **B**. ice cream with agraz.

are comparable with the ones obtained in this study, in which viscosity ranged from 1,814±12.9 mPa s (@ 6 rpm, 5 °C, splinde 6) for the control sample to 2,513±8.05 mPa s (@ 6 rpm, 5 °C, splinde 6) for the ice cream added with Andean blueberry powder. These results agree with those reported by Hwang et al. (2009), who showed changes in the rheological properties of an ice cream added with grape wine lees, and the reduction of the melting rate due to the presence of carboxy methyl cellulose and grape wine lees, which could absorb water and increase the viscosity. In the current study, the increase of the viscosity resulted in a lower overrun than that observed in the control sample (Table 1). It is expected that an ice cream with a low overrun has less air content within the structure, and the product becomes harder. The addition of Andean blueberry powder had a significant effect (P<0.05) on the enthalpy and ice melting temperature (T_f) values. Both decreased in the ice cream formulated with Andean blueberry, reaching values of about 78.86 J g^{-1} and -4.27 °C, respectively (Table 1).

Table 1. Techno functional properties of the ice cream.

	Ice cream with Andean blueberry powder	Control sample
Viscosity (mPa s) (@ 6 rpm, 5 °C)	2513±8.05^a	1814±12.9^b
Melting temperature (°C)	-4.27±0.71^a	-2.79±0.84^a
Enthalpy (J g^{-1})	78.86±2.54^b	103.70±1.05^a
freezable water (%)	21.43±2.85^b	31.76±0.32^a
Overrun (%)	36.01±1.78^b	41.79±0.99^a
Melting time (min)	18.23±0.69^a	12.05±0.65^b
Melting rate (%)	23.47±0.63^b	26.25±0.84^a

Similarly, the addition of Andean blueberry decreases the amount of freezable water in the ice cream, likely due to its water-holding capacity (Suresh et al. 2017). As can be seen in Table 1, the control sample had a higher percentage of freezable water (31.76±0.32%) than that observed in the ice cream added with Andean blueberry powder (21.43±2.85%). This behavior is in agreement with the results previously reported by Hwang et al. (2009), who suggested that the decrease in enthalpy is due to a lower freezable water content in the sample. In contrast, Ullah et al. (2015) increased the antioxidant capacity of ice cream, by adding different percentages of sugarcane juice. However, due to the increase in the amount of water, they obtained a high value of overrun and low viscosity. The addition of Andean blueberry had a significant effect (P<0.05) on the melting time, with a higher value (18.23±0.69 min) than the one obtained in the control sample (12.05±0.65 min). The ice cream with Andean blueberry experienced a reduction in the melting rate of 23.47±0.63% compared to the control sample of 26.25±0.84% (P<0.05). Aboulfazli et al. (2014) suggest that an increase in the sample viscosity causes a decrease in the melting rate. Other additives, such as emulsifiers, when the concentration is increased, cause a delay in the melting time and a low melting rate. Thus, all these findings suggest that Andean blueberry led to an increase in the water-holding, increasing the viscosity, decreasing the overrun, and delaying the melting time. Hence, the Andean blueberry powder could be considered as a natural potential stabilizer.

Stability

Table 2 shows the values of phenol compounds, antioxidant capacity, color, and texture (hardness) obtained for ice cream during storage. The hardness significantly increased (P<0.05) during the storage time, in both the ice cream added with Andean blueberry powder and the control sample, where this behavior could be associated with the decrease in overrun, caused by a lower resistance of the matrix because it contains air within its structure. It should be noticed that the considered good quality ice cream has a hard texture compared to that produced with low-fat content, mainly due to the low overrun. Regarding the color, the L*, a*, and b* parameters showed no statistically significant differences (P>0.05) during the storage time

(Table 2). In contrast, the control sample had significant differences ($P<0.05$) in the b* parameter, showing an increase in the intensity of yellow during storage time. In the current study, the total phenolic compounds had no significant changes ($P>0.05$) during storage time (Table 2). This result is in agreement with that reported by Ścibisz and Mitek (2007), who conducted a stability study at -15 °C for Highbush blueberries (*Vaccinium Corymbosum* L). Likewise, the antioxidant capacity of ice cream measured by the ABTS*+ and DPPH methods had no significant changes ($P>0.05$) during storage time, with values between 22.57 and 25.14 µmol TEs 100 g^{-1} sample by the ABTS*+ and DPPH method, respectively (Table 2). Sharma et al. (2015) improved the antioxidant capacity in an ice cream formulation, by adding wine lees, obtaining values, using the DPPH test, of 1.95 mg of Trolox g^{-1}. Nascimento et al. (2018) reported a high content of total phenols and antioxidant capacity in ice cream added with 2% grape flour, with values of 142.03±10.59 µmol Trolox g^{-1}. The antioxidant capacity depends on several factors, such as the formation and stability of radicals, and the location of antioxidants in the matrix during the different phases of processing. Hence, the concentration and source of the antioxidant, either pulp or dry product, is essential to achieve the highest content of both phenols and the antioxidant capacity. Nevertheless, the increase in the antioxidant capacity can increase the final cost of the product.

Table 2. Stability study of ice cream.

	Ice cream with Agraz			Sample control		
Time (days)	t=0	t=15	t=30	t=0	t=15	t=30
Hardness (N)	19.11±1.24^c	21.61±0.22^b	23.35±1.12^a	15.11±0.49^e	17.29±0.79^d	19.13±0.20^c
Color						
L*	63.32±0,36^b	64.63±0.88^b	65.65±2.09^b	80.31±0.41^a	80.92±0.68^a	82.7±0.45^a
a*	6.33±0.42^a	5.92±0.81^a	6.62±0.68^a	-1.15±0.10^b	-1.07±0.13^b	-1.01±0.09^b
b*	5.97±0.81^d	6.18±0.17^d	6.26±0.37^d	13.76±0.01^c	15.12±0.41^b	16.41±0.69^a
Total phenols (mg GAE 100 g^{-1})	80.28±1.19^a	80.49±1.54^a	80.85±2.00^a	n.d	n.d	n.d
ABTS^{+}* (µmol TEs 100^{-1} g dry mass)	22.57±1.39^a	22.88±1.48^a	22.83±1.38^a	n.d	n.d	n.d
DPPH (µmol Trolox 100^{-1} g dry mass)	25.48±1.03^a	25.07±1.90^a	25.14±0.68^a	n.d	n.d	n.d

Results expressed as mean (n = 3) ± standard deviation. Different lowercase letters in the row indicate significant differences between treatments. n.d = not detected.

CONCLUSION

The incorporation of Andean blueberry powder into ice cream presents a viable strategy for enhancing both the nutritional and functional attributes of Ice cream. The drying process, while causing a reduction in total phenolic compounds and antioxidant capacity compared to the fresh pulp, still allows the powder to significantly improve the ice cream's overall stability and health benefits. The observed increase in viscosity, decrease in overrun, and delayed melting time suggest that the powder acts as a stabilizer. Furthermore, the retention of phenolic compounds and antioxidant capacity in the final product, even after storage, highlights the potential of Andean blueberry powder as a functional food ingredient. The enhancement of the nutritional profile of ice cream without negatively impacting its sensory attributes or stability presents an opportunity for the formulation of desserts that align with consumer preferences. Future research should investigate the optimal levels of agraz powder incorporation into ice cream formulations to achieve the desired sensory attributes and nutraceutical benefits without compromising product stability.

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Evaluation of two milk replacers in the artificial rearing of Anglo-Nubian female goat kids

UNIVERSIDAD NACIONAL DE COLOMBIA

Evaluación de dos lacto-reemplazantes en la crianza artificial de cabritas Anglo-Nubian

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ABSTRACT

Keywords:
Artificial feeding
Dairy goats
Growth
Preweaning period
Production costs

Dairy goat production is an alternative for rural development in Buenos Aires province, Argentina. Artificial rearing with milk replacers allows all goat milk to be intended for milking. Although this technology is widespread in cattle, more studies are needed in goats. The aim was to evaluate the artificial rearing of Anglo-Nubian female kids, according to two milk replacers: commercial small ruminant milk replacer (MR) vs. whole cow milk powder (MP). Milk replacers were offered until week 10 of life according to a protocol, complimenting it with solid food after week three. The following were evaluated: survival (SUR), live weight (LW), daily live weight gain (DLWG), age (week) in which the kids reached 10 kg (LW10) and multiplied by 2.5 their birth weight (BWx2.5), glycemia (GLY), diarrhea incidence (DI), and cost of milk replacer per kid (COST). SUR was 100% in both treatments. Despite detecting differences in LW in week six in favor of MP, both groups reached similar LW at the end of the rearing period. Results for DLWG, LW10, BWx2.5, and DI did not differ between treatments. There was no effect of treatment on GLY; however, age affected it. COST was lower for MR. It is concluded that, although both milk replacers allow similar and adequate growth of kids, the use of a milk replacer formulated for small ruminants significantly reduces the cost of artificial rearing.

RESUMEN

Palabras clave:
Alimentación artificial
Cabras lecheras
Crecimiento
Período predestete
Costos de producción

El tambo caprino es una alternativa productiva para el desarrollo rural en la provincia de Buenos Aires, Argentina. La crianza artificial con lacto-reemplazantes permite destinar toda la leche al ordeñe. Si bien esta tecnología está ampliamente difundida en vacunos, son necesarios más estudios en caprinos. El objetivo fue evaluar la crianza artificial de cabritas Anglo-Nubian según dos lacto-reemplazantes: sustituto lácteo formulado para rumiantes menores (MR) vs. leche en polvo entera vacuna (MP). Los lacto-reemplazantes fueron ofrecidos hasta la semana 10 de vida según protocolo, complementándose a partir de la semana 3 con alimento sólido. Se evaluó: supervivencia (SUR), peso vivo (LW), ganancia diaria de peso vivo (DLWG), semana en que las cabritas alcanzaron 10 kg (LW10) y multiplicaron por 2,5 su peso al nacimiento (BWx2,5), glucemia (GLY), cuadros diarreicos (DI) y costo del lacto-reemplazante por cabrita (COST). SUR fue del 100% en ambos tratamientos. Pese a detectarse diferencias en el LW en la semana seis a favor de MP, al final del período de crianza, ambos grupos alcanzaron similar LW. Tampoco se encontraron diferencias entre los tratamientos para DLWG, LW10, BWx2,5 y DI. No hubo efecto del tratamiento sobre GLY, aunque hubo efecto de la edad. COST fue menor para MR. Se concluye que ambos lacto-reemplazantes permiten un crecimiento similar y adecuado de las cabritas. Sin embargo, la utilización del sustituto lácteo formulado para rumiantes menores disminuye significativamente los costos de la crianza artificial.

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In Argentina, goat production is mainly developed in arid areas, under extensive conditions, for meat and milk in the north and fiber in the south (Martínez and Suárez 2018). In recent years, goat production began to develop out of its traditional areas in more intensive systems. In Buenos Aires province, goat stock has been increasing, reaching 273% in 2008-2017 (Ghibaudi et al. 2018) and mainly intended to dairy. Such production is facilitated by the agroecological characteristics of this province (humid temperate climate, with mean temperatures from 7-11 °C to 20-25 °C, and average annual rainfall of 800 mm) (DPE 2020) and its proximity to major consumer centers. This activity has several advantages that could contribute to rural development: a) it can be performed in small areas (small farmers); b) it is usually carried out with family labor, which would promote rural settlement; c) it can be related to rural tourism ; d) it allows smallholders to generate value-added products, for example, goat milk can be processed in the farm; e) both goat meat and milk and their derivatives have benefits for healthily human nutrition.

In goat dairy farms, rearing kids naturally with their dams or artificially with goat milk represents a high opportunity cost due to the decrease in milk production in the industry. Therefore, artificial rearing based on milk replacers has been proposed. In addition, to allowing all the milk to be intended for milking, artificial rearing offers other advantages: improved growth of triplets and quadruplets, uniformed weaning groups, accelerated ruminant transition, better health control of kids, etc. (Castroalonso et al. 2003; Quintana Quiñonez 2018). However, its results depend on several factors (environment, nutrition, genetics, health, management, etc.) (Balasopoulou et al. 2022; Vickery et al. 2022), so it is necessary to develop accurate protocols.

The duration of artificial rearing depends on the production objective. When comes to obtain replacement females, it is usually extended up to three months (Chacón-Hernández and Boschini-Figueroa 2015). However, it can be as short as 45 days if animals are intended for slaughter (Tacchini et al. 2006). Another important factor is cost, so other criteria, such as target live weight or a proportional increment of birth live weight, rather than age, have been proposed for weaning (Datt et al. 2023).

The concentration and composition of milk replacers are crucial for artificial rearing success. In Buenos Aires province, goat dairy farms have been effectively using cow milk powder due to its advantages, e.g., its high-quality, easily digestible nutrients, and physical form, which simplifies storage (Simonetti et al. 2019). However, it has a high-cost input as competes with its use for human consumption; furthermore, its price is affected by the export market. The presence of a milk replacer formulated for small ruminants in the local market is recent and there's a lack of studies that demonstrate its benefits.

This study aimed to compare the performance of a milk replacer formulated for small ruminants with whole cow milk powder in the artificial rearing of Anglo-Nubian female goat kids.

MATERIALS AND METHODS

The study was performed following the etic requirements of the Institutional Committee for Care and Use of Experimental Animals (CICUAE) of the Facultad de Ciencias Agrarias (FCA), Universidad Nacional de Lomas de Zamora (UNLZ).

Location

The assay was developed in the "Small Ruminants Teaching, Training and Research Module" (FCA, UNLZ), municipality of Lomas de Zamora, located in the metropolitan area of Buenos Aires province, Argentina (34°47'18"S and 58°26'56"W).

Experimental animals

Twenty Anglo-Nubian female goat kids were used. At birth, they were separated from their dams, identified with ear tags and weighted. During their first 48 hours, they were fed colostrum obtained by hand milking and administered through nursing bottles.

Housing

During the day, the animals were housed in a 35 m^2 outdoor pen surrounded by woven wire and provided with a covered area, water troughs and feeders (Figure 1A).

At night, confinement was done in a room equipped with boxes measuring 1.4 m^2 each, where animals were grouped in four (Figure 1B). The boxes had slatted floors with a separation of 1 cm between wood strips, to prevent contact with urine and feces. During the first week, the slated floor was covered with wood shaving to help keeping body temperature.

Figure 1. Daytime outdoor pen (A) and night indoor boxes (B).

Daily, the boxes were cleaned and disinfected with quaternary ammonium, and the wood shaving was changed. The outdoor pens were regularly sprinkled with lime to prevent diseases.

Treatments

Female goat kids were assigned to one of two treatments based on their birth weight:

Small Ruminant Milk Replacer (MR; n=10): Fed with Ternelac Plus milk replacer formulated for small ruminants (Ducrem S.A., Argentina), at the recommended concentration for goat kids of 150 g L^{-1}. This product has 88.0% dairy components, including skim milk, whey protein and lactose; its non-dairy components are mainly deactivated whole soybean flour.

Whole Cow Milk Powder (MP; n=10): Fed with La Sorianita whole cow milk powder (San Satur S.A., Argentina) at a concentration of 150 g L^{-1}.

The chemical composition of both milk replacers is provided in Table 1.

Table 1. Chemical composition of milk replacers used for artificial rearing of Anglo-Nubian female goat kids.

Parameter	MR*(2)	MP**
Crude protein (min)%	25.0	26.1
Total fat (min)%	25.0	26.4
Crude fiber (max)%	0.50	0.00
Lactose (max)%	36.0	38.5
Metabolizable energy (Mcal kg^{-1})(1)	4.65	5.03
Ash (max)%	9.00	4.00
Calcium (max)%	1.20	1.10
Phosphorus (min)%	0.65	0.77
Moisture (max)%	4.65	4.86

*Small ruminant milk replacer.
**Whole cow milk powder.
(1)Metabolizable energy was calculated from information provided by the manufacturers, based on Yeom et al (2002)
(2)Additives: minerals (copper, zinc, iron, magnesium, manganese, iodine, selenium and cobalt), vitamins (B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12} and D_3) colorants and flavorings.

Feeding and management

Feeding was carried out following a gradual transition from colostrum to milk replacer during the first three days. Thereafter, feeding followed the scheme of Table 2.

Table 2. Feeding scheme used for artificial rearing of Anglo-Nubian female goat kids.

Week	Liquid diet scheme		Solid feed scheme		
	* L d^{-1}	Daily feeding shifts	Alfalfa hay	Starter feed[(1)]	Alfalfa pellets
1**	0.5 to 0.75 L d^{-1}	2	-	-	-
2	1 L d^{-1}	2	-	-	-
3[(2)]	1 L d^{-1}	2	Ad libitum	Ad libitum	-
4	1.2 L d^{-1}	2	Ad libitum	Ad libitum	-
5	1.5 L d^{-1}	2	Ad libitum	Ad libitum	-
6	1.5 L d^{-1}	2	Ad libitum	Ad libitum	-
7	1.5 L d^{-1}	2	Ad libitum	Ad libitum	-
8	1 L d^{-1}	2	Ad libitum	Limited to 300 g	Ad libitum
9	0.75 L d^{-1}	1	Ad libitum	Limited to 300 g	Ad libitum
10	0.5 L d^{-1}	1	Ad libitum	Limited to 300 g	Ad libitum

*Liquid diet (small ruminant milk replacer or whole cow milk powder) 150 g L^{-1}.
**A gradual adaptation of goat kids to the consumption of their respective milk replacers was carried out, starting from goat's milk. During the first six feeding shifts in the following proportions of goat's milk/milk replacer were used: 75-25%; 50-50%; 25-75%.
(1)Composed by 18.0% minimum crude protein, 3.0% minimun total fat, 7.5% minimun crude fiber, 8.8% maximun ash, 0.8% minimun calcium, 0.6% minimun phosphorus and 2,700 kcal kg^{-1} metabolizable energy.
(2) Goat kids began to be offered drinking water.

As shown, feeding was done twice a day (800 and 1,700 h) until the end of week eight and thereafter, once a day during the afternoon feeding shift. Both milk replacers were prepared with potable water at 50 °C for better dissolution and supplied at 37 to 42 °C. Initially, they were administered with nursing bottles and later on, with individual buckets equipped with a plastic probe connected to a nipple for goat kids (Figure 2). Nipples were placed in an upper position to allow the passage of the milk replacer by suction and not by gravity, to reduce losses due to dripping.

Figure 2. Milk replacer supply system (bucket with teat).

From the third week, the diet was completed with starter feed for early weaned calves (Marcelo E. Hoffmann e Hijos S.A.) and alfalfa hay. The starter feed was offered ad libitum until an average daily consumption of 300 g per animal, when it was restricted, and alfalfa pellets were incorporated ad libitum. Alfalfa hay was always available. They had also access to drinking water ad libitum.

Sampling and records

Survival rate (SUR; %): Rate of live kids at the end of the tenth week of artificial rearing.

Live weight (LW; kg): Weight at birth and then once a week until the tenth week. Measurements were taken in fasting state (in the morning, before milk intake, and without access to liquids and solids for at least 12 h), using a digital scale with a precision of 10 g.

Daily live weight gain (DLWG; g day^{-1}): Daily weight gain during rearing. It was calculated by dividing the difference between the final (10th week) and the initial weight (birth weight) by the elapsed period (70 days).

Reaching 10 kg LW (LW10): Age in weeks when all goat kids in each group reached 10 kg.

Multiplying their birth weight by 2.5 (BWx2.5): Age in weeks when all goat kids in each group reached a weight 2.5 times their birth weight.

Glycemia (GLY; mg dl^{-1}): Concentration of glucose in plasma. Samples were taken every two weeks from week four. A disposable syringe and a 21 G (25x8) needle were used to extract 2 mL of blood from the jugular vein. The collected volume was placed in an Eppendorf tube containing 20 µL of EDTA solution supplemented with potassium fluoride as an anti-glycolytic agent to preserve samples from glycolysis (anticoagulant G, Wiener Lab). The samples were centrifuged at 3,500 rpm for 15 min. Then, plasma was separated, transferred to 0.5 mL Eppendorf tubes, and stored at -20 °C until analysis. Plasma samples were sent to a private laboratory (Dorronsoro Laboratory, Animal division. Las Flores, Buenos Aires, Argentina) for analysis by spectrophotometry using Mindray BS-200 equipment.

Diarrhea incidence (DI; %): Proportion of goat kids that presented diarrhea disorders and duration of the episodes. Immediately before milk replacer administration, the ano-caudal region of each goat kid was observed to identify signs of diarrhea.

Cost of milk feeding per goat kid (COST; US$ kg^{-1}): Cost of both milk replacers relative to the number of weaned goat kids in each treatment at week ten. The prices on July 2023 were 3.39 US$ kg^{-1} and 5.68 US$ kg^{-1} for the milk replacer for small ruminants and whole cow milk powder, respectively, both placed in the processing plant.

Statistical analysis

LW was analyzed by PROC MIXED for repeated measurements, according to the treatment (MR vs. MP), the age (weeks 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10), and its interaction. The structure of the variance-covariance matrix, selected following the Akaike criterion, was a heterogeneous autoregressive matrix of order 1. Tukey's test was used for multiple comparisons. Variables related to the target weights (LW10 and BWx2.5) and SUR were evaluated using Fisher's exact test according to the treatment. Effect of treatment on DLWG was evaluated by PROC GLM. GLY was analyzed using PROC MIXED for repeated measurements, including the treatment (MR vs. MP), the age (weeks 4, 6, 8, and 10), and its interaction. The matrix structure selected, following the Akaike criterion, was autoregressive of order 1. Tukey's test was used for post-hoc comparisons. COST was calculated as a partial direct cost of using each milk replacer per weaned goat kid. All analyses were performed using SAS statistical software (2023).

RESULTS AND DISCUSSION

Effect on survival rate (SUR)

The survival rate of kids at weaning was 100% for both treatments. These results are consistent with those previously obtained by Simonetti et al. (2019) and by other authors (Galotta 2018; Arias et al. 2022) with the same breed, under similar conditions. Feeding with a milk replacer for goat kids offered ad libitum (Delgado-Pertíñez et al. 2009a, 2009b) also resulted in 100% survival for Florida and Payoya breeds when rearing was done until four weeks of age.

Among the factors that determine the survival of artificially reared kids are the number of animals as well as the uniformity of the group at the start of rearing. Castroalonso et al. (2003), in a trial conducted with a high number (n=354) of replacement kids selected from the Canary Islands Group, obtained a high mortality rate (25.0%) using a specific milk replacer for goat kids and feeding through nursing bottles twice a day. According to these authors, the determining factors were the age differences and the low weight at the start of artificial rearing for half of the animals. In their work, the main causes of detected mortality were: malnutrition, due to inability to adapt to the feeding system; colibacillary diarrhea, due to deficient colostrum intake; pneumonia, due to environmental factors; and enterotoxemia, resulting from overconsumption of feed. Regarding commercial dairy goat farms, a survey conducted by Correa (2006) in Buenos Aires (Argentina) stated that mortality during artificial lactation was 27.2%. According to the results found by Balasopoulou et al. (2022) in Germany, the lack of individual control of colostrum intake, the inability to identify health problems and early weaning with inappropriate weights, are the main factors that decrease survival.

In this trial, the use of bedding made of wood shaving, together with collective housing in pens, could have contributed to prevent deaths due to hypothermia. Another important requirement for the correct management of artificial rearing is the intensive dedication of operators.

Effect on live weight (LW) and daily live weight gain (DLWG)

When analyzing weight evolution (Table 3), an interaction between age (week) and treatment (MR and MP) was detected (P<0.01). LW in MP was higher only in week six (P<0.05). At the end of artificial rearing, both treatments showed a similar LW (P>0.10), with a difference of only 180 g, representing less than 1.30% of the average live weight.

DLWG throughout artificial rearing was 153 and 157 g for MR and MP, respectively, not resulting in significant differences (P>0.10).

Table 3. Live weight evolution (LW; kg) in Anglo-Nubian female goat kids fed with small ruminant milk replacer (MR) or whole cow milk powder (MP) (lsmeans (min.-max.); SEM: standard error of the mean).

Age (weeks)	Treatment		SEM	*P*-value
	MR	MP		
0	3.20 (2.72-3.87)	3.17 (2.68-3.71)	0.148	0.800
1	3.78 (3.30-4.36)	3.83 (3.48-4.25)	0.146	0.798
2	4.53 (4.15-5.19)	4.82 (4.20-5.25)	0.182	0.267
3	5.41 (4.81-5.99)	5.69 (5.09-6.27)	0.146	0.174
4	6.45 (5.86-7.49)	6.79 (6.26-7.08)	0.165	0.151
5	7.91 (7.20-9.32)	8.24 (7.76-8.57)	0.199	0.253
6	9.03 (8.27-10.48)	9.60 (8.91-10.2)	0.193	0.037*
7	10.4 (9.17-12.1)	10.9 (9.69-12.0)	0.268	0.239
8	11.6 (10.5-13.6)	11.7 (10.1-12.9)	0.294	0.821
9	12.9 (11.6-15.9)	13.0 (10.6-14.2)	0.381	0.960
10	13.9 (12.3-17.5)	14.1 (10.7-15.4)	0.470	0.739

*Indicates statistical differences between treatments (P<0.05).

The results regarding weight-related variables are affected by aspects such as sex, breed, milk replacer composition and duration of rearing period. DLWG obtained in this study are comparable to those indicated as acceptable for replacement kids by Bach et al. (2010). Galotta (2018), working with Anglo-Nubian kids fed with powdered milk up to eight weeks of age, obtained 143±8.4 g day^{-1}, thus reaching a weaning weight of 10.4 kg. In the present study, LW at week eight was 11.6 kg for MR and 11.7 kg for MP, and DLWG was 150 g day^{-1} in MR and 153 g day^{-1} in MP.

In a previous study by Simonetti et al. (2019), feeding goat kids of this breed with calf milk replacer or whole cow milk powder, both at 13.8% dry matter, registered weights of 12.5 and 13.7 kg, respectively, at week 10, resulting significantly different; this could be due to the low-fat content of the calf milk replacer. However, when rearing was extended until week 12, similar weights were achieved (14.4 and 14.8 kg), probably due to compensation through solid feed consumption. In their recent study, Arias et al. (2022) compared the growth of kids of the same breed fed with calf milk replacer and milk replacer for small ruminants, used at 18.0% up to week six; they found a non-significant difference in DLWG (129 vs. 137 g day^{-1}, respectively), but a tendency in favor to the specific milk replacer. Their results are similar to that found in this work, where DLWG at week six for MR was 138 g day^{-1}. It is interesting to note that in the mentioned study the milk replacer was offered in a higher concentration, but this did not lead to better DLWG.

Delgado-Pertíñez et al. (2009a, b) evaluated the live weight of Florida and Payoya kids raised with their mothers versus ad libitum supply of a milk replacer formulated for small ruminants (23.7% crude protein and 25% total fat) at 17% w/w, obtaining 7.41 and 6.99 kg for the mentioned breeds, respectively, at four weeks of age. These results are higher than those obtained in the present study for MR at week four (6.45 kg). Delgado-Pertíñez et al. (2009a, b) pointed out that live weights were higher in artificial rearing for the Florida breed and in natural rearing for Payoya. These results indicate that, in artificial rearing, it may be useful to adjust the amount of dry matter of the replacer to that of the dam's milk, at least when working with dual-purpose breeds, such as Anglo-Nubian. Differences between Delgado-Pertíñez et al. (2009a, b) and the present study may be due, among other factors, to the breed and the way of supply (ad libitum or not).

Regarding general recommendations, the dry matter content of milk replacer will influence the volume consumed. Liquid consumption during artificial rearing is approximately 25% of live weight up to four weeks of age and 15.0% of live weight thereafter (Quintana Quiñonez 2018). A suitable milk replacer for kids should contain a high proportion of dairy protein (close to 90%) instead of vegetable sources, 20.0 to 28.0% protein and 16.0 to 24.0% fat (Martínez and Suárez 2018).

The partial substitution of dairy by vegetable protein in milk replacers is commonly made to reduce costs; however, this can affect the growth rate. In this study, although the presence of soybean meal in the specific small ruminant milk replacer, no effect on live weight was found. Additionally, according to Yeom et al. (2002), the protein-to-fat ratio in the milk replacer should be taken into account, because it may affect protein retention in the animal's body, which in turn affects weight gain. When the energy supply is limited, part of the protein provided by the milk replacer is used as an energy source, decreasing the efficiency of protein utilization for growth. These authors found a significant effect on average daily live weight gain, when the crude protein to metabolizable energy ratio was high (equivalent to 46.7 g kcal^{-1}), partially attributable to the increased consumption of milk replacer offered ad libitum. This ratio was even higher in the present trial, corresponding to 53.76 and 53.70 g kcal^{-1} for MR and MP, respectively.

Effect on reaching 10 kg LW (LW10) and multiplying their birth weight by 2.5 (BWx2.5)

All goat kids in both treatments reached 10 kg at week eight. The average age when the kids achieved this weight was 47.5 days for MR and 45.7 days for MP. Atasoglu et al. (2008), working in a partial natural lactation system, lasted 55 days to reach 10 kg.

All goat kids in MR and MP reached the objective of multiplying by 2.5 their birth weight (BWx2.5) at week six. This coincides with Ugur et al. (2004) who, working with Saanen kids, did not find significant differences in body measurements between groups weaned at 45 or 60 days, indicating that weaning could take place at six weeks as long as growth objectives are reached. It is important to note that in this trial, only 20.0% of the goat kids in MR registered this value at week five of age, vs. 70.0% in MP, which allows to consider earlier weaning when using powdered milk, as long as other conditions, e.g. adequate solid food intake, are met. The average age when kids reached BWx2.5 was 36.4 days for MR and 33.6 days for MP, making this criterion earlier than LW10.

Gökdal et al. (2017), evaluating Saanen and Alpine kids in partial suckling systems and supplementing with milk replacer, managed to triple the birth weight at 42 days, without differences between breeds. Galotta (2018), using Anglo-Nubian kids, obtained the same percentage of live weight gain compared to birth weight (55.0%)

when using whole cow milk powder (150 g L^{-1}) and goat milk in artificial rearing until 24 days of age. In the present trial, the weight gain proportions at that moment (estimated on the weights of weeks three and four) were 81.1% for MR and 94.0% for MP.

Among the criteria to determine the weaning time are age, weight, and average daily intake of solid food. Weaning criteria used in commercial goat farms are varied. A survey conducted by Vickery et al. (2022) worldwide agrees that the main weaning criterion used is age (72.9%), followed by the target weight. In this survey, 22% of farmers used both criteria. According to Gökdal et al. (2017), live weight is a more accurate criterion than age to wean.

Datt et al. (2023) indicate that weaning at 10 rather than 7 kg would lead to better growth later on. So, the proportion of kids reaching 10 kg at the end of rearing was one of two target weights evaluated in the present study. However, the above-mentioned authors, stated that, due to the great number of factors that may affect growth (genetic, maternal nutrition, sex, litter size, feeding, etc.), it might not be suitable to use a fixed weight or age to define weaning time; instead, other parameters like body condition score or growth based on birth weight could be more appropriate. For this reason, the multiplication of birth weight by 2.5 proposed by Datt et al. (2023) was used in this study.

Effect on glycemia (GLY)

Regarding GLY, no interaction between treatment and week was detected (Table 4). There were also no significant differences between treatments (90.7 vs. 94.2 mg dl^{-1} for MP and MR, respectively) (P>0.10). However, it was affected by age, with a decrease observed through the weeks.

Table 4. Glycemia evolution (mg dl^{-1}) in Anglo-Nubian female goat kids fed with small ruminant milk replacer (MR) or whole cow milk powder (MP) (lsmeans (min.-max.); SEM: standard error of the mean).

Age (Weeks)	Lsmeans (min.–max.)	SEM	*P-value*
4	98.3 (84.0-131)^{a}	1.85	0.0009
6	93.2 (81.0-106)^{b}	1.83	
8	90.6 (80.0-110)^{bc}	1.88	
10	87.6 (74.0-99.0)^{c}	1.85	
Treatment			
MR	94.2 (78.0-106)	1.82	0.190
MP	90.7 (74.0-131)	1.82	
Treatment x Age			0.266

Different letters indicate statistical differences between weeks, given the media of treatments (P<0.05).

The results obtained in this study differ from those of Simonetti et al. (2019), who found lower glycemia from week six onwards for Anglo-Nubian kids fed with either powdered milk or calf milk replacer. Galotta (2018), working with Anglo-Nubian kids raised with cow milk powder, found 110 and 89.3 mg dl^{-1} in weeks 6 and 9, respectively. Tacchini et al. (2006) evaluated the response of Saanen x Criollo kids to a commercial milk replacer, finding blood glucose values (close to 98.0 mg dl^{-1}) on day 25 comparable to those of the present study at 28 days. Similarly, Aufy et al. (2009), reported 97.0 mg dl^{-1} of glycemia at this age. Paez Lama et al. (2014) did not detect a difference in glycemia between criollo kids raised by their dams vs. artificially reared, but they did detect an age effect, similar to which was found in this study.

At birth, the function of the ruminant digestive system resembles that of non-ruminants. The transition to a functional rumen is accompanied by anatomical and physiological changes, including colonization of the rumen by fermentative bacteria, anatomical development of rumen papillae and modification of endogenous metabolism from glycolytic to glycogenic (Baldwin et al.

2004). The absence of differences in glycemia obtained herein between treatments suggests that the changes in the metabolic pathways regarding to the energy source had similar development in both of them.

Smith and Sherman (2009) indicate that the normal range of blood glucose in adult goats is between 50.0 and 75.0 mg dl^{-1}, with an average of 62.8 mg dl^{-1}. According to these authors, values outside this range can indicate gastrointestinal problems. In the present study, the kids did not reach normal values in either of the two treatments at the end of the trial. This contrasts with the results previously obtained (Simonetti et al. 2019), where normal range values were already found at week six and the kids had blood glucose levels close to the mean (67.7 mg dl^{-1}) at week eight. According to Abbasi et al. (2012), kids from four months of age have blood glucose values corresponding to those of adults and do not show variations related to the energy level of the diet. Aufy et al. (2009) working with Saanen kids found that the evolution of glycemia tends to depend more on the weaning management, such as the reduction of milk replacer volume offered or the introduction of weaning solid mixtures, than on the milk replacer itself.

Effect on diarrhea incidence (DI)

The incidence of diarrhea was not significant in any treatment. For MR, diarrheal cases were detected in weeks two (two kids, 20.0%) and four (one kid, 10.0%). In MP, the presentation was similar, with diarrhea detected in weeks two (one kid, 10.0%) and four (two kids, 20.0%). Their durations were very brief, as symptom remission was observed by the next feeding shift or the next day. These results are better than those of a previous study (Simonetti et al. 2019), with DI in more animals when a milk replacer formulated for calves was used. Such difference could be attributed, at least partially, to the fact that the non-specific milk replacer, generally formulated with a considerable amount of whey, contains a high proportion of lactose, that can cause intolerance in goat kids. Furthermore, unlike such previous trials (Simonetti et al. 2019), DI was not related to the transition from colostrum to milk replacer or to an increase in volume intake.

Murray et al. (2008) detected severe cases of diarrhea until four weeks of age and lasting up to 15 days in Boer and Boer crossbred kids fed milk replacer (for both, calves and small ruminants); these diarrheas affected daily gain, not reaching the target weight of 15 kg for weaning at 84 days. However, kids fed either by their dams or artificially with goat or cow milk presented mild diarrhea, lasting one to three days, which did not affect weight gain.

The “Neonatal Diarrhea Complex” appears from the interaction among etiological agents, animal’s immune status, and rearing management factors (hygiene, housing density) (Smith and Sherman 2009). It is relevant to note that, typical of an intensive system with susceptible categories, the hygiene of facilities and implements is crucial for reducing diarrheas caused by pathogens. As previously detailed in methodology, the hygiene guidelines adopted in the present assay (box cleaning, wood shaving change and lime sprinkled) in addition to the facility’s design to keep kids away from other categories, seem to have been efficient. However, diarrheas can occur in the absence of enteric pathogens. They can be caused by physicochemical factors that alter the osmotic balance during intestinal absorption; among them, excessive intake or incorrect reconstitution of milk replacer can affect abomasal renine activity so that the components of the replacer would not reach the intestine under optimal conditions for degradation and subsequent absorption (Martínez and Suárez 2018). Moreover, simple indigestion, acute carbohydrate consumption, copper deficiency and intoxications might generate diarrheas (Smith and Sherman 2009).

Effect on the cost of milk feeding per goat kid (COST)

The consumption of milk replacer per weaned kid was 10.7 kg in each treatment. Thus, the cost of the milk replacer per weaned kid (COST) was US$ 36.29 for MR and US$ 60.79 for MP. These values imply a cost difference of 67.5% in favor of MR.

If the results obtained regarding the target weights are considered, weaning could be anticipated by 2 weeks in the case of LW10 and by 4 weeks in the case of BWx2.5, without impacting the subsequent development of the goats (Datt et al. 2023). This would reduce milk replacer costs by 12.3% for both treatments in the case of LW10 and by 33.9% in the case of BWx2.5. Additionally, the possibility of weaning at 5 weeks could be taken into account in the case of MP, due to the high proportion of goats that reached BWx2.5 at this point, which would reduce costs by 48.7%. In such a case, it would be advisable to combine this weaning criterion with, for example, the level of solid food consumption, to reduce the probability of post-weaning weight loss.

Paez Lama et al. (2013) reported that artificial rearing of criollo kids up to 45 days old was not economically viable, being 80.4% higher than natural lactation. The authors found that these results were due to two factors: the increase in labor costs and the lower price per liter of marketable goat milk compared to milk replacer. Tacchini et al. (2006) indicated that a commercial milk replacer for small ruminants was 154% more expensive per weaned Saanen x Criollo crossbred kid than a self-formulated replacer based on goat whey, cow milk powder, brewer's yeast and fish meal. In contrast, Delgado-Pertíñez et al. (2009a, b) found that the economic outcome of using a replacer for small ruminants improved the margins of dairy systems in Payoya and Florida breeds.

Taking into account that dairy goat farms in Argentina are commonly of small scale, so depending on family labor, it is important to highlight that feeding represents the most important cost in artificial rearing systems. Cow milk powder is a high-cost input since it competes with its use for human consumption; Furthermore, it is an important exportable product and its price and availability are affected by the international market.

CONCLUSION

In dairy goat farms, artificial rearing with milk replacers allows all goat milk to be intended for the market. In Argentina, although this technology is widespread in cattle, more studies are needed in goats. In alignment with such directive, the aim was to evaluate two milk replacers, a commercial small ruminant milk replacer and whole cow milk powder, for artificial rearing of Anglo-Nubian female kids. Taking into account the lack of differences in all evaluated variables (survival, growth related measures, glycemia, and diarrheas), the small ruminant milk replacer should be recommended, with the advantage of reducing the cost associated with liquid feeding. This study intends to help with the development of more accurate and economically viable feeding protocols, which could be further adopted by small ruminant farmers.

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Physicochemical and mechanical characterization of *Brosimum lactescens* and *Parkia discolor* wood from Guaviare, Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

Caracterización físicoquímica y mecánica de la madera *Brosimum lactescens* y *Parkia discolor* del Guaviare, Colombia

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ABSTRACT

Keywords:
Bending
Density
Holocellulose
Lignin
Parallel compression

Colombia has numerous wood species of great importance; however, information on many of these species is scarce. For this reason, the woods Guaimaro (*Brosimum lactescens*) and Dormidero negro (*Parkia discolor*) cultivated in Calamar (Guaviare) were studied, with the aim of determining their properties and enhancing their rational use. The evaluations were carried out following the Colombian Technical Standards NTC 290, 663, 701 and 784. Density was evaluated according to NTC 290; shrinkage and stability coefficient were determined following the NTC 701; the holocellulose content was according to the procedure of Wise, the lignin content and extractives were determined as described by TAPPI T 222 and T 204; the mechanical bending strength with NTC 663 and to calculate the parallel compression under the NTC 784. The Guaimaro is a very dense wood, dimensionally stable and of high resistance to bending and parallel compression being of optimal use for construction with extractives of 18.52%, holocellulose of 65.59% and lignin of 35.81%; it presents higher lignin contents than other species of the same genus. The Dormidero negro has a medium density, low dimensional stability, and low resistance to static bending and parallel compression. It is not suitable for construction, but it can be used for carpentry. The chemical composition was consistent with that of other species in the same genus, with extractives at 15.88%, holocellulose at 69.30%, and lignin at 31.77%. Expanding mechanicaltests, such as hardness and shear tests, is recommended, along with exploring treatments to enhance its properties.

RESUMEN

Palabras clave:
Flexión
Densidad
Holocelulosa
Lignina
Compresión paralela

Colombia cuenta con numerosas especies maderables de gran importancia; sin embargo, la información de muchas de estas especies es escasa. Por ello, se estudiaron las maderas Guaimaro (*Brosimum lactescens*) y Dormidero negro (*Parkia discolor*) manejadas en Calamar, Guaviare; con el objetivo de determinar sus propiedades y propender por su uso racional. Se evaluó la densidad siguiendo la Norma NTC 290; las contracciones y coeficiente de estabilidad se determinaron con la Norma NTC 701; los contenidos de holocelulosa mediante el procedimiento de Wise, el contenido de lignina y los extractivos se determinaron como se describe en TAPPI T 222 y T 204; la resistencia mecánica a flexión con la Norma NTC 663 y compresión paralela Norma NCT 784. El Guaimaro es una madera muy densa, muy estable dimensionalmente y de alta resistencia a la flexión y compresión paralela, siendo de óptimo uso para construcción; con extraíbles de 18,52%, holocelulosa de 65,59% y lignina de 35,81%; presenta mayores contenidos de lignina que otras especies del mismo género. El Dormidero negro presentó mediana densidad, poco estable dimensionalmente, de baja resistencia a la flexión estática y a la compresión paralela, no aptas para construcciones, si para la ebanistería; la composición de contenidos químicos fue igual a otras especies del mismo género, extraíbles 15,88% holocelulosa 69,30% y lignina 31,77%. La composición química fue coherente con la de otras especies del mismo género, con un 15,88% de extractivos, un 69,30% de holocelulosa y un 31,77% de lignina. Se recomienda ampliar las pruebas mecánicas, como las de dureza y cizallamiento, y estudiar tratamientos para mejorar sus propiedades.

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Brosimum lactescens is a native species to the region known as the “Atlantic Forest” which is located along the southeastern end of Brazil, it grows over 2,700 meters above sea level (masl) and, because to this long altitudinal gradient, both temperature and precipitation have very wide spectra throughout the territory, therefore, the mean annual temperature varies between 20 and 25.1 °C (Carneiro 2014), while the average annual rainfall is between 1,000 and 4,000 mm depending on the area. Given these factors, it is possible to identify areas of endemism in this region (Lazos et al. 2017). In addition to the above, it has been distributed throughout northern South America thanks to the Amazon, so it has been able to reach out to countries such as Bolivia, Colombia, Peru and Venezuela (dos Santos et al. 2020), even going to Central America where the species has been sampled, especially in Costa Rica (Quesada-Monge and Fernández-Vega 2012), therefore, it may occur in humid tropical forests and very humid tropical forests (dos Santos et al. 2020). When they pass to dominate the canopy, their height usually ranges from 18 to 35 m, being common to be close to 30 m. Leaves are simple, alternate, elliptic to oblong. Fruits are sub globose drupes about 1-2 cm in diameter, yellow or orange when ripe, simple, alternate (Spichiger et al. 1989), acuminate apex, it presents exudate both in leaves and stem, which is white and abundant (Flores 2014). It is known by the vulgar name of Chimicua or White Palisangre in Peru (Villacorta 2011; Flores 2014). Quecho in Bolivia (Poorter et al. 2001) and Ojoche in Costa Rica (Quesada-Monge and Fernández-Vega 2012). The seeds can be eaten boiled, roasted, or fried. It is also used milled to make tortillas.

Their fruits support the diet of the avifauna, and their leaves and sap are used in folk medicine. It also presents good potential to be used as an ornamental plant, as well as it is widely used in places where it lives by the production of latex, fodder resources, folk medicine and the production of its fruits, in addition, it has significant properties that give high value to its timber and non-timber products, so it is included as species of potential value of sustainable exploitation (Minorta-Cely et al. 2019).

Parkia discolor is a native species to South America, in Venezuela, Colombia, Peru and northern Brazil. Its habitat is the Amazon rainforest, in open areas on floodplains on poor, white and sandy soils of the Amazon rainforest (Pereira and Ferreira 2010; Guariguata and Kattan 2002). It has managed to make way in the Amazon Forest so it is possible to be found in lands adjacent to the Amazon basin, such as the Atlantic Forest and the Brazilian. This species is found in the Orinoco River basin in Venezuela and Colombia (Ramos and Varela 2003), extending along the gradient there present, thus offering a suitable model for the study of germination behavior in response to water availability. Pereira and Ferreira (2010) mention that it is one of the species of the genus *Parkia* with 15 m in height. Leaves are compound and alternate; inflorescence showy, of capilliform type, and its horizontal axis projects beyond the foliage; its fruits in the form of pods are oblong, coriaceous, and indehiscent and contain between 9 and 15 seeds. It is a species that largely tolerates flooding; therefore, it is capable of remaining underwater for long periods. That said, it requires sites with low disturbances to develop correct functioning and growth (Pereira and Ferreira 2010). This species does not have a common name because it is not commonly found; however, in Colombia, it is known as Dormidero (Galindez and García-R 2011), while in Venezuela it is called ´Casabe de murciélago´ (Vidondo 2014), on the other hand, in many texts, mainly of Portuguese language refer to this principally ´visgueiro-do-igapó´, like other variations such as araratucupi and faveira, although these are ways of calling other species of the genus *Parkia* (Hopkins 1986; Pereira and Ferreira 2010; Pereira and Ferreira 2017). It can be used in a range of ways, ranging from timber to non-timber products, stands out as gum, due to its fruits having similar properties to those found in gum Arabic, and also has medicinal characteristics that have been used by various communities (Ahmed 2018); finally, it has ornamental and ecological potential (Ramos and Varela 2003). At the industrial level, wood is considered a vital resource used in the building sector, pulp for paper, biomaterial production and clean energy generation. In this context, the importance of characterizing the chemical properties of wood is due to the direct impact that its basic composition has on its ability to retain water as well as their characteristics of flexibility, strength and rigidity. These aspects are fundamental for determining the behavior of wood in various industrial applications, enabling its optimal use and ensuring performance under diverse conditions. Natural forest wood is therefore a renewable natural resource of great ecological and economic importance for many regions around the world. In forest ecosystems, wood is the main structural component and performs various functions in the provision of ecosystem services such as soil and water conservation, carbon capture and storage, and biodiversity conservation (Fichtner and Härdtle 2021).

In recent years, interest in the study and marketing of new and lesser-used timber species from natural forests has increased to reduce over-exploitation and pressure of more traditional and demanded species; this makes poorly studied woods potential substitutes for high-value tropical species (Sseremba et al. 2011). In this sense, the communities of Puerto Cubarro in Calamar (Guaviare) manage the woods of *Brosimum lactescens* and *Parkia discolor* in a sustainable way by using extraction techniques that do not affect the health of the forest. With controlled harvesting and the implementation of appropriate management practices, which ensures the preservation of ecosystems, avoiding deforestation and promoting long-term sustainability.

From this perspective, knowledge of wood in terms of its physical properties, such as shrinkage and density, is essential for a sustainable and efficient use of this resource (Dong et al. 2016). Shrinkage of wood, which is the change in wood dimensions according to moisture content is one of the most important parameters to consider in the design and manufacture of wood products in order to eliminate future defects and unstable dimensions (Sargent 2019). Wood density, on the other hand, is a key indicator of wood quality in terms of mechanical strength and load capacity, which determines its suitability to be used in different structural applications and moved in the global market (Ramage et al. 2017).

Proper knowledge of the contraction and density of these new species is crucial to developing sustainable forest management techniques, establishing criteria for the classification and selection of woods, and optimizing their industrial and commercial processing. Through a solid understanding and application of knowledge about these fundamental physical properties of wood, these vital ecosystems can be protected and conserved and at the same time, facilitate the development of related industries and the promotion of a circular and sustainable economy (Arriaga et al. 2023).

Thus, the aim of this study was to characterize the wood by determining density, shrinkage, resistance to bending and compression parallel to fibers; as well as holocellulose, lignin and wood contents of *Brosimum lactescens* and *Parkia discolor* species cultivated in Calamar (Guaviare) region.

MATERIALS AND METHODS

Materials

The woods were extracted from the Puerto Cubarro sidewalk (1°50 32” N, 72°42 40.2” W) in the municipality of Calamar located on the left bank of the Unilla River belonging to the Department of Guaviare, 80 km from San José del Guaviare the capital with a mean annual precipitation of 2,800 mm, mean temperature up to 26.5 °C, and relative humidity >80% (Figure 1).

Ten trees of each species of *Parkia discolor* and *Brosimum lactescens* were used; all the activities of directed logging and of preparation for their extraction from the forest were included. Felling operations involved cutting the standing tree, measurement to determine the ideal size of the logs, limbing and bucking. Its fall was directed in order to reduce damage to the remaining mass and thus ensure forest sustainability. Good fuel management was ensured in all harvesting operations to avoid spills that altered soil conditions or caused a fire.

The logs were sawn and dimensioned for the extraction of samples or wooden specimens that after an airdrying process were measured and tested to technologically characterize the species under study. The test pieces were elaborated following the methodology proposed by the American Society for Testing and Materials (ASTM 2003) and the Colombian technical standards (NTC), which establish the procedures both in specifications of the test pieces, test speed and number of samples to be tested.

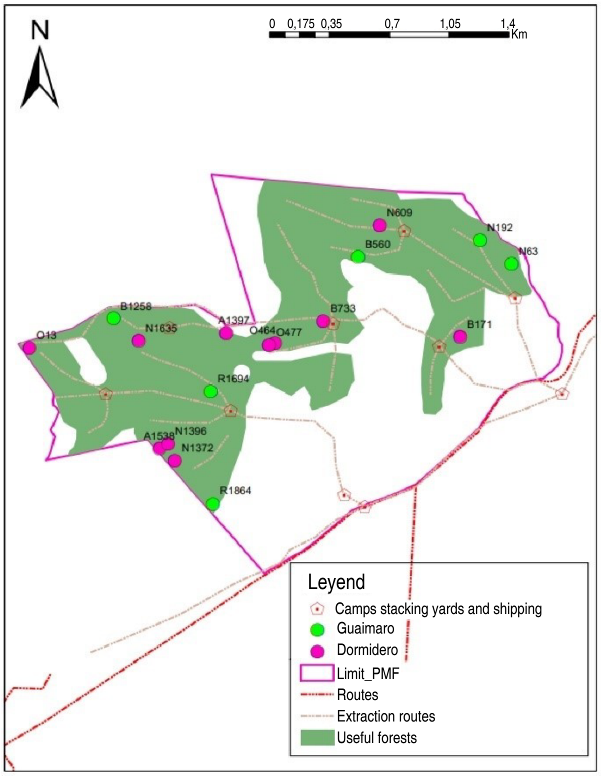

Figure 1. Location map of the trees under study, Puerto Cubarro, in the Calamar region (Guaviare).

From each log, a central plank 8 cm thick was taken for the length of the log, from which two lumbers were obtained, maintaining very well-differentiated radial and tangential orientations, from which the test pieces necessary for each of the tests were obtained (Figure 2).

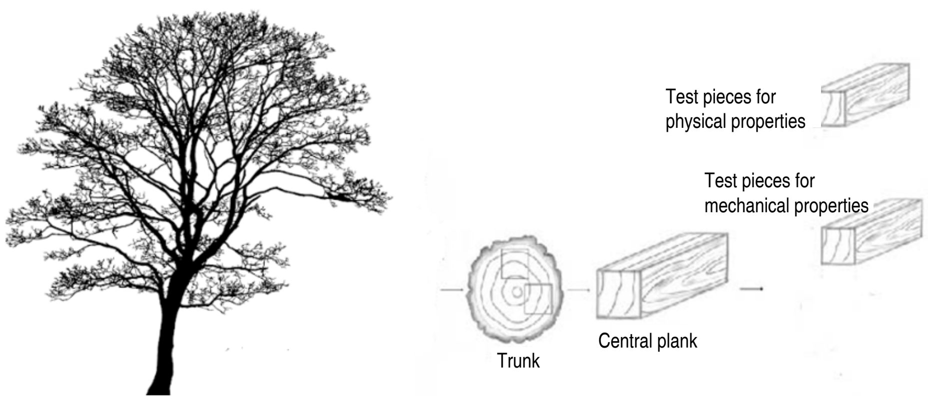

Figure 2. Scheme of wood extraction.

For the physical properties, 20 test pieces per species with a dimension of 3x3x10 cm length were used. To determine wood density under different moisture content conditions, the Colombian Technical Standard NTC 290 (ICONTEC 2006) was followed and to determine the radial, tangential, longitudinal and volumetric contractions of the wood following the NTC 701.

Mechanical properties characterization

For the mechanical properties, 20 test pieces were extracted with dimensions of 5x5x20 cm, as established by the Colombian Technical Standard NTC 663 (NTC 2006), and 2.5x2.5x41 cm according to NTC 784 (NTC 2006), to calculate the parallel compression and static bending tests of the two forest species, which were free of defects such as knots, piths, cracks, missing edges, etc.

To evaluate the mechanical properties, the wood was taken to a dry-air (moisture content close to 12%). The tests were carried out in a Tinius Olsen universal testing machine with a load capacity of 15,000 kilograms.

Determination of chemical composition

The holocellulose content was determined using sodium chlorite according to the procedure of Wise et al. (1946). In a beaker, 150 mL of 1.5% (w/w) sodium chlorite solution were poured into 2 g of wood, and 10 drops of glacial acetic acid and the sample was placed in a water bath at 75 °C for 1 h. After that hour, 10 drops of glacial acetic acid and 1.5 g of sodium chlorite were added, it was cyclically repeated every hour, for a total period of 4 h. After chlorination, the solution was filtered, washed with 200 mL of cold water, followed by 10 mL of acetone; the residue was taken to a conventional oven at 105 °C until constant weight.

The lignin content was determined as described by TAPPI T 222 om-02 (2002). 15 mL of 72% sulfuric acid (w/w) were added to 1 g of wood sample, stirring it for 10 min and letting it stand for 2 h. Subsequently, 575 mL of distilled water were added and it was boiled for 4 h maintaining a constant volume, if necessary distilled water was occasionally added. Finally, it was filtered in Büchner funnels and the samples were repeatedly washed until the acid residues were removed. Finally, samples were brought to constant weight in an oven at 105 °C. All measurements were made in 12 replicates for each chemical composition determination (one sample per tree). Data were presented as percentages on a dry basis.

The extractive content was determined as described by TAPPI T 204 CM-97 (1997). 100 mL of NaOH to 1% were added to 2 g of wood sample, stirring it with a glass rod for 10 seconds, and placed the sample in a water bath, letting the water in the boiling bath for 1 h (97 °C); after bath, it was stirred again for 25 minutes. The digestion material was filtered and neutralized by adding 25 mL acetic acid to 10% for 1 min, then it was washed with 100 mL of hot water. The previous step was repeated, with a second portion of 25 mL of acetic acid to 10% for 1 min, then washed again with hot water leaving the material free of acid. Finally, they were brought to constant weight in an oven at 105 °C.

Data analysis

The statistical analysis was performed using a completely randomized experimental design for each test, with the help of the Statgraphics software. The information was processed according to the methodology described by Hoshmand (2006).

RESULTS AND DISCUSSION

Physical properties

The results of density, dimensional stability coefficient (DSC) obtained for Guaimaro (*Brosimum lactescens* (S. Moore) C.C. Berg) *are presented in* Table 1 and for Dormidero negro (*Parkia discolor* Spruce ex Benth) in Table 2.

The results of the physical properties appear in each box and are represented as follows:

$\bar{y}$	$\pm$	q
	CVt	

Where, $\bar{y}$ is Mean, ± q is 95% Confidence Interval and CVt is the total coefficient of variation.

Table 1. Physical properties of the wood *Brosimum lactescens* (S. Moore) C.C. Berg species.

	Green (MC:45%)	Air-dry (MC:14%)	Anhydrous	Basic
Density (kg m^{-3})	1,048.28±21.27 4.33%	938.22±20.03 4.56%	898.85±20.33 4.83%	760.96±19.52 5.48%
	Tangential	**Radial**	**Volumetric**	**Dimensional Stability Coefficient (DSC)**
Shrinkage				
Total (%)	9.64±42.96	7.55±0.14	17.91±0.27	1.28±0.029
From green to oven-dry	9.09%	4.02%	3.27%	4.81%

Table 2. Physical properties of the wood *Parkia discolor* Spruce ex Benth species.

	Green (MC:45%)	Air-dry (MC:14%)	Anhydrous	Basic
Density (kg m^{-3})	750.11±53.28 15.17%	640.00±63.67 22.71%	605.22±66.46 23.46%	540.61±55.55 21.56%
	Tangential	**Radial**	**Volumetric**	**Dimensional Stability Coefficient (DSC)**
Shrinkage				
Total (%)	6.06±0.41	2.62±0.16	8.86±0.4	2.36±0.21
From green to oven-dry	14.61%	13.29%	11.32%	19.42%

The Guaimaro is considered a high-density wood due to the value obtained in dry air density of 938.22 kg m^{-3} and is classified as a heavy wood thanks to the anhydrous density value obtained of 898.85 kg m^{-3}; values that are within the range suggested by the ASTM (760-1,000 kg m^{-3}). According to the basic density classification of the NTC 2500 (*Civil Engineering and Architecture*. Using *Wood* for *Construction*), the wood of *Brosimun lactescens* species belongs to Group A, making it useful for construction with 760.96 kg m^{-3} (greater than 710 kg m^{-3}). These results are equal to those obtained by Rosales-Solórzano et al. (2018) in studies made for the same wood. Still, they are higher than those found for the wood of *Brosimun alicastrum* species, which has medium density and belongs to the Group B category used for construction purposes (Escobar and Rodríguez 1993).

The wood of Guaimaro (*B. lactescens*) has a high total volumetric shrinkage of 17.91%, placing it in the range suggested by the ASTM (15-20%). The wood has a very stable dimensional stability coefficient (DSC) of 1.28% (less than 1.5%), which determines its suitability for construction.

The Dormidero Negro is a wood of medium density and moderately heavy, with a dry air density of 640.00 kg m^{-3} and an anhydrous density of 605.22 kg m^{-3} range suggested by the ASTM (510-750 kg m^{-3}). The wood of *Parkia discolor* belongs to Group C, it is considered not suitable for heavy constructions and high resistance with 540.61 kg m^{-3} (400-550 kg m^{-3}). These results were similar to those reported by de Miranda et al. (2012) and were higher than those obtained in *Parkia biglobosa* wood (Ataguba et al. 2015).

The wood of Dormidero negro (*P. discolor*) presents a low total volumetric shrinkage of 8.86%, placing it in the range suggested by the ASTM (less than 10%). The wood is dimensionally unstable with a Dimensional Stability Coefficient (DSC) of 2.36% (greater than 1.8%); which makes it unsuitable to be used as a raw material for construction. These results are equal to those obtained for the wood of *Parkia biglobosa* (Ataguba et al. 2015).

Mechanical properties

The results of the mechanical characterization of the wood Guaimaro (*Brosimum lactescens*) species are presented in Table 3 and Table 4 for the wood of Dormidero negro (*Parkia discolor*).

The response of Guaimaro wood to static bending is considered to be of a slightly high resistance according to ASTM standards with a modulus of rupture of 1,293.16 kg cm^{-2} (1,260-1,519 kg cm^{-2}) and slightly high elasticity with MOE of 168.41x10^3 kg cm^{-2} (156-185 kg cm^{-2}), superior to that reported by Escobar and Rodríguez (1993) of 142x10^3 kg cm^{-2} for the wood of the species *Brosimun alicastrum*.

Table 3. Mechanical properties of the wood Brosimum lactescens species.

Condition	Static bending			Compression parallel
	ELP	MOR	MOEx10^3	MOR
	(kg cm^{-2})			
Air-dry 12%	890.02±12.92 13.10%	1293.16±62.09 10.26%	168.41±19.48 24.72%	786.82±25.13 16.82%

Table 4. Mechanical properties of the wood *Parkia discolor* Spruce ex Benth species.

Condition	Static bending			Compression parallel
	EPL	MOR	MOE x10^3	MOR
	(kg cm^{-2})			
Air-dry 12%	197.58±12.79 13.82%	713.50±67.47 20.21%	51.02±4.75 19.83%	92.24±6.73 15.58%

The response of the wood of Guaimaro (*B. lactescens*) to stress resistance in compressions parallel considers the wood to have high resistance to such compressions according to ASTM standards with compression parallel of 786.82 kg cm^{-2} (750-1,049 kg cm^{-2}), superior to that reported for the same wood of the species *B. alicastrum* of 725 kg cm^{-2} (Escobar and Rodríguez 1993). It can be used as wooden roofs, columns and pillars, wooden beams, wood for structures, etc.

The wood of Dormidero negro (*Parkia discolor*) presents low resistance to static bending with a modulus of rupture of 713.50 kg cm^{-2} (754-510 kg cm^{-2}) and very low elasticity with MOE of 51.02x10^3 kg cm^{-2} (43-71 kg cm^{-2}) according to ASTM standards, lower than those reported for *Parkia gigantocarpa* and *Parkia biglobosa* wood (de Miranda et al. 2012; Ataguba et al. 2015).

The response of Dormidero negro (*P. discolor*) wood is of very low resistance to compression parallel to the grain of 92.24 kg cm^{-2} (less than 479 kg cm^{-2}) according to ASTM standards, equal to those reported for *P. gigantocarpa* wood (de Miranda et al. 2012) and less than those obtained for *Parkia biglobosa* wood (Ataguba et al. 2015. It could be used to manufacture boxes or packaging that are not exposed to high pressures. It can also be employed in modular construction systems with low structural load and in decorative elements, such as picture frames, mirrors, or ornaments, where aesthetics and ease of handling are prioritized over strength.

Chemical composition

The composition of the polysaccharides obtained for the Guaimaro (*Brosimum lactescens*) wood and Dormidero negro (*Parkia discolor*) are listed in Table 5.

Table 5. Results for the chemical composition of the woods of *Brosimun lactescens* and *Parkia discolor* species.

	Holocellulose (%)	Lignin (%)	Extractives (%)
Brosimun lactescens	65.59±3.19	35.81±5.27	18.52±4.35
	8.96%	5.91%	9.45%
Parkia discolor	69.30±2.51	31.77±3.55	15.88±3.55
	7.45%	4.50%	9.07%

The holocellulose contents for Dormidero negro wood were similar to those reported for *P. pendula* and *P. timoriana* woods (Wahyuni 2018; Batista et al. 2021). The amount of lignin was similar to those reported for *P. pendula* and *P. timoriana* woods (Wahyuni 2018; Batista et al. 2021).

The wood of Guaimaro presented low contents in the polysaccharides formed by holocellulose of 65.60%, which were lower than those reported for the wood *B. alicastrum* of 81.05% (Castañeda 1986). Regarding lignin contents, they were 35.81% higher than those reported for *B. alicastrum* wood of 20.44% (Castañeda 1986).

The percentage of extractives for Guaimaro (*Brosimun lactescens*) wood was on average 18.52%, similar to that obtained for *Brosimum alicastrum* (17.51%), in studies conducted by Castañeda (1986) following the same procedure.

The percentage of extractives for Dormidero negro (*Parkia discolor*) wood is comparable to that presented by Batista et al. (2021); but greater than those obtained for *Parkia timoriana* wood (Wahyuni 2018).

CONCLUSION

The Guaimaro (*Brosimum lactescens*) is a wood of very good dimensional stability. It is a high-density wood classified as hardwood; it belongs to Group A, meaning that it can be used for construction purposes. It has high resistance to different bending strengths and compression parallel. This wood contains higher lignin content than other species in the same genus.

On the other hand, the wood of Dormidero Negro (*Parkia discolor*) presents very low dimensional stability. The wood of medium density; belongs to Group C undesirable for heavy constructions, but it can be used for carpentry. It presents low responses to mechanical properties. In terms of chemical composition, they are equal to other species in the same genus.

To expand the knowledge base, further investigations are recommended on their response to conventional drying treatment and alternative methods, such as thermal modification for the woods of Guaimaro (*Brosimum lactescens*) and Dormidero negro (*Parkia discolor*), which can modify their internal structure and thus improve a greater dimensional stability and hydrophobicity of these woods.

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The growth and yield of cocoyam (*Colocasia esculenta* (L.) Schott) as affected by storage methods

UNIVERSIDAD NACIONAL DE COLOMBIA

Crecimiento y rendimiento del cocoyam (*Colocasia esculenta* (L.) Schott) afectados por los métodos de almacenamiento

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ABSTRACT

Cocoyam (taro) (*Colocasia esculenta*) is an important tropical crop that requires minimal inputs compared to yam. However, increasing its production to meet the growing population's food demand is hindered by the susceptibility of planting materials to rotting in the field before the next season. While reports on storage conditions for cocoyam cormels are available, information on the storage of corms (propagules) remains limited. Hence, this study assessed simple storage methods and the field performances of corms stored under these methods at the Ayepe research field of the University of Ibadan, Ibadan, Nigeria in 2019. Corms stored under shade, in pits, and on raised platforms were evaluated in a completely randomized design with three replicates. On the field, freshly harvested corms (S1), corms stored under shade (S2), corms stored in pits (S3) and corms stored on raised platforms (S4) were evaluated in a randomized complete block design with three replicates. The results indicated that weight loss and storage efficacy differed significantly ($P<0.05$) among the storage conditions. Weight loss ranged from 8.95 (S2) to 29.87% (S4), while storage efficacy ranged from 71.20 (S4) to 91.20% (S2). Corm emergence was significantly higher in S2 compared to S4 at 2 and 4 weeks after planting but was similar to the other treatments. Propagule storage conditions had no significant influence on cocoyam growth and yield. However, cormel yields for S1, S2, S3 and S4 were 7,483, 6,625, 6,729 and 6,208 kg h^{-1}, respectively. Corms stored under shade or in pits were, therefore, recommended.

Keywords:
Corm storage
Emergence
Growth
Taro
Weight loss
Yield

RESUMEN

El cocoyam (taro) (*Colocasia esculenta*) es un importante cultivo tropical que requiere unos insumos mínimos en comparación con el ñame. Sin embargo, el aumento de su producción para satisfacer la creciente demanda de alimentos de la población se ve dificultado por la susceptibilidad de los materiales de siembra a pudrirse en el campo antes de la siguiente temporada. Aunque existen informes sobre las condiciones de almacenamiento de los cormos de cochayama, la información sobre el almacenamiento de los cormos (propágulos) sigue siendo limitada. Por lo tanto, este estudio evaluó métodos de almacenamiento simples y el rendimiento de campo de los bulbos almacenados con estos métodos en el campo de investigación Ayepe de la Universidad de Ibadan, Ibadan, Nigeria en 2019. En el campo, se evaluaron cormos recién cosechados (S1), cormos almacenados bajo sombra (S2), cormos almacenados en fosas (S3) y cormos almacenados en plataformas elevadas (S4) en un diseño de bloques completos al azar con tres repeticiones. Los resultados indicaron que la pérdida de peso y la eficacia del almacenamiento difirieron significativamente ($P<0,05$) entre las condiciones de almacenamiento. La pérdida de peso osciló entre 8,95 (S2) y 29,87% (S4), mientras que la eficacia de almacenamiento varió entre 71,20 (S4) y 91,20% (S2). La emergencia de cormos fue significativamente mayor en S2 en comparación con S4 a las 2 y 4 semanas después de la siembra, pero fue significativamente mayor en S2 en comparación con S4, similar a los otros tratamientos. Las condiciones de almacenamiento de los propágulos no tuvieron influencia significativa sobre el crecimiento y rendimiento del cocoyam. Sin embargo, los rendimientos de cormel para S1, S2, S3 y S4 fueron 7.483, 6.625, 6.729 y 6.208 kg h^{-1}, respectivamente. Por lo tanto, se recomendó almacenar los bulbos bajo sombra o en fosas.

Palabras clave:
Almacenamiento de cormos
Emergencia
Crecimiento
Taro
Pérdida de peso
Rendimiento

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Cocoyam [*Colocasia esculenta* (L.) Schott] is a common crop cultivated in the tropics and subtropics, and consumed as a vegetable and tuber crop. The swollen underground stem of cocoyam is called the corm, while the smaller offshoots from the corm are called cormels (Castro et al. 2005). The cormel has relatively high starch content, minerals, vitamin C, niacin thiamine, and riboflavin, better than cereals (Mitharwa et al. 2022). Although yams have greater cultural significance, cocoyam has more agronomic advantages and limited input requirements. Therefore, the crop is relatively cheaper, and the production is limited to small-scale farmers. Nigeria is the world's largest cocoyam producer, with an estimated 3.20 and 3.21 million metric tons annually from 0.81 and 0.82 million hectares of land in 2020 and 2021, respectively (FAO 2022). Despite the increase in production and land area, the yield of cocoyam in Nigeria decreased from 39,771 kg h^{-1} in 2020 to 39,408 kg h^{-1} in 2021. The reduction in yield per unit area can be attributed to storage challenges faced by small-scale and resource-limiting farmers, who are Nigeria's primary contributors to cocoyam cultivation (Mukaila et al. 2022). One of the major constraints of cocoyam production has always been the availability of vegetative propagule for the expansion of the crop (Owusu-Darko et al. 2014).

Cocoyam is propagated vegetatively through corms that can be sourced locally from previous harvests. After harvest, the corms are left on the field and quite a large amount get rotten before the next season, leading to a shortage of propagation materials (Opara 2003). Consequently, some cormels are used as planting material for field establishment or expanding crop cultivation for more income. Using cormels as a propagule for field establishment in cocoyam reduces the sufficiency of food supply and farmers' income (Mukaila et al. 2022). Another attempt to obtain adequate vegetative materials for planting is through micropropagation or tissue culture but this method is expensive for small-scale farmers with limited resources for cultivation (Quain et al. 2018). These resource-limited farmers require an affordable means by which the corms for propagule can be stored after harvest. According to Opara (2003), aside from the traditional method of heaping corms under shade for storage, suspending corms tied into bundles with the attached basal petiole is common in the South Pacific and this is not a common practice in Nigeria. Also, using special structures for corms storage in cocoyam cultivation has not been a common practice, but rather for cormel storage. Information on the loss or damage of cocoyam propagules in storage is limited. Most reports on *Colocasia esculenta* storability have been for the cormels (Eze et al. 2015; Mugumo 2021).

The effect of storage conditions on tannia (*Xanthosoma sagittifolium*) and taro cormels (*Colocasia esculenta*) has been reported (Mugumo 2021). However, the method that was reported as most appropriate could be inappropriate for taro under varying conditions, due to their differences in moisture content and variation in skin toughness against storage pests and diseases may cause differences in the rate of rotting in storage (Sajeev et al. 2004; Oyefeso et al. 2021). According to Opara (2003), tannia has a lower respiration rate than taro cormels when the temperature in storage increases. Consequently, taro rotten faster in storage than tannia when not properly monitored. Similarly, the performances of corms stored under different conditions could differ in establishment and growth rate due to variations in the condition of corms at planting. Hence, there is a need to assess different inexpensive and efficient storage methods for taro vegetative propagule to improve crop production by resource-limited farmers. This study aimed to determine the easily accessible on-farm methods of storing taro corms for propagule and evaluate these propagules for variation in field performance.

MATERIALS AND METHODS

Site Description

The study involved storage and field experiments conducted in 2019 at the Ayepe research station of the Department of Agronomy, University of Ibadan, at Isokan Local Government Area, Osun State, Nigeria. The coordinates of the location were Latitude 7.288029°N and Longitude 4.284788°E. The vegetative descriptions at the location were reported by UN-Habitat (2014), while the prevailing relative humidity, temperature, and rainfall were obtained from NASA POWER project (Table 1).

Table 1. The weather conditions at the experimental site in 2019.

	Temperature at 2 meters (°C)	Relative Humidity at 2 meters (%)	Precipitation Corrected (mm/day)
January	23.13	70.81	0.00
February	26.00	84.62	0.00
March	26.07	85.88	5.27
April	25.82	87.69	0.00
May	25.47	90.94	5.27
June	24.75	91.38	5.27
July	24.48	89.94	5.27
August	24.37	89.81	5.27
September	24.51	90.62	5.27
October	25.26	90.06	5.27
November	25.34	84.62	0.00
December	22.55	63.25	0.00
Annual average	24.80	84.94	5.27

NASA POWER project (2024).

Treatments and Experimental Design

The treatments for the storage conditions involved corms stored under shade, corms stored in pits, and corms stored on raised platforms evaluated for three months (January to April) in a completely randomized design with three replicates. The treatments for the field experiments (from April to December) were the establishment of the corms collected from freshly harvested corms (S1) and the various storage conditions (corms stored under shade (S2), corms stored in pits (S3) and corms stored on raised platforms (S4) carried out in a randomized complete block design with three replications.

Storage Experiment

Cocoyam corms of 150 to 200 g that were healthy, and without wounds or any physical primary injuries were separated and bulked for the different storage methods study. The S1 corms were collected from the research field of the Department at Ayepe, while the S2 corms were stored under the shade of cassia trees. A pit of 1 m in length, 1 m breath, and 0.5 m deep was constructed and corms were placed at the bottom of the pit, then covered with palm frond for the S3 storage condition. Raised platforms for the S4 were erected from bamboo sticks, one meter high above ground level. The corms were covered with palm fronds in all the storage conditions, except for the freshly harvested corms. The freshly harvested corms were the undisturbed corms collected from under cocoa and kola nut trees as practiced by farmers and planted directly at the time of field establishment.

Field Establishment and Management

The vegetation was manually cleared, and the refuse was removed from the plot marked out for the study. Each plot size was 5 m x 5 m per plot and 1 m between plots. Heaps were manually constructed at 1 m x 1 m apart. From the stored corms, good corms (150 to 200 g each) were selected as planting materials from each of the storage methods on 26/4/2019. Planting was done by planting one corm per heap. One plant per heap was maintained to reduce overcrowding by regularly rouging out every other offshoot from the main stalk. Weeding operations were manually carried out on the field at 4, 8, and 12 weeks after planting. Subsequently, weeding of the plot was carried out, when necessary.

Data Collections

Data were collected on corm weight loss in storage after three months of storage by measuring the corm weight before and after storage for each storage condition and expressing the result in percentages (Equation 1). The storage efficiency was calculated by determining the percentages of the good corms after storage (Equation 2). The storage loss and storage efficiency were determined after three months (January - April 2019) in storage.

$$\text{Weight loss (\%)} = \frac{\text{Weight of corm at storage} - \text{Weight of corm after storage}}{\text{Weight of corm at storage}} \text{ x } 100 \quad (1)$$

$$\text{Storage Efficacy (\%)} = \frac{\text{Number of corms at storage} - \text{Number of corms after storage}}{\text{Weight of corms at storage}} \text{ x } 100 \quad (2)$$

The rate of corm emergence was determined at 2, 4, 6 and 8 weeks after planting (WAP). Cocoyam height (the tallest petiole of the leaves that stand erect from the underground corm) was measured using a ruler. The stem diameter (the base of the leaves petiole from the underground corm) was determined using a Vernier caliper starting from the 12th WAP and continued at monthly intervals for seven months. Harvesting of the corms and cormels were done 9 months after planting as practiced by farmers in the locality. At harvest, corm and cormel length (using a ruler) and diameter (using a Vernier caliper) were measured. The weight of corms ha^{-1}, the number of cormels ha^{-1} and cormel ha^{-1} were determined using the Camry dial scale model SP-20.

Data Analysis

The observed data were subjected to descriptive statistics and analysis of variance and the significantly different means were separated using Least Significant Difference (LSD) at $P<0.05$.

RESULTS AND DISCUSSION

Corms weight loss and the efficiency of different storage conditions

Weight loss in taro corm was significantly ($P<0.05$) affected by the storage conditions (Table 2). The corm stored under S2 had the least weight loss, while the corm stored in S4 had the highest weight loss. Weight loss during storage in cocoyam (aroids) like every other root and tuber crop has always been attributed to moisture loss, physical damage, and deterioration resulting from physiological activities and rodent attacks (Ubalua et al. 2016; More et al. 2019). This loss in weight conforms with Eze and Nwani (2014) report that tannia lose more weight in storage than taro, due to the higher moisture content in tannia than taro. A similar result was reported by USAID and CIP (2015), which stated that storage in the pit was more appropriate for sweet potatoes than the other traditional methods due to minimal deterioration through moisture loss, sprouting, and pathological losses.

Table 2. Influence of storage on corms weight loss and the efficiency of the storage conditions.

Storage methods	Weight loss (%)	Storage efficacy (%)
Freshly harvested propagule (S1)	-	-
Propagule stored under shade (S2)	8.95	91.20
Propagule stored in pits (S3)	15.43	81.60
Propagule stored on raised platforms (S4)	29.87	71.20
LSD	4.26	5.51

The storage efficiency varied significantly ($P<0.05$) for storage methods and had a similar trend with the weight loss observed (Table 2). Storing cocoyam corms under S2 gave the highest storage efficiency and retained the number of corms stored by 10.52 and 29.92% more than S3 and S4, respectively. Also, the least efficient method for storing corm is S4. The finding conforms with Behailu et al. (2023) report that using a raised platform for storing cocoyam corm resulted in propagule with the lowest quality of the storage methods evaluated. The inefficiency of the storage method is attributed to a higher rate of evaporation and transpiration that leads to increased rotting and loss of stored materials (Eze and Ameh 2011). According to Baidoo et al. (2014) and Diaguna et al. (2023) rotting of taro in storage starts after two weeks of storage. The time spent by the corms under the different conditions was long enough to facilitate an enormous level of rotting of the corms. For the corms to have stayed three months under the various storage conditions, the pathogens responsible for rotting would have ample time to cause more damage than necessary (Eze and Ameh 2011). The loss of 17.5 (corm) and 40.6% (cormel) under prolonged storage have also been reported by Diaguna et al. (2023). The observed results indicated that the use of S4 in storing propagules was most inefficient compared to the other methods, while the efficiency of S2 was the highest.

Emergence of cocoyam as influenced by corm storage methods

The emergence of the corms obtained from different storage conditions is shown in Figure 1. The corms obtained from S2 had significantly (P<0.05) higher emergence than the S4 at 2 WAP but were similar to the other treatments. While the corms from the other storage condition had less than 50% emergence at 2 WAP, the S2 treatment had over 70%. At 4 WAP, the corm emergence for S1, S2, and S3 were significantly higher than the S4 treatments. The delay in the emergence in S4 may result in the poor performance of crop raise from this storage method. A similar result was reported by Behailu et al. (2023) that storing corms on raised platforms reduced sprouting in cocoyam corms. The result is in support of Finch-Savage and Bassel (2016) report. According to their report the delay in emergence results in poor growth. They were able to report the relationship between weight loss and crop emergence. The poor emergence is attributed to higher weight loss that could have resulted from evapotranspiration and respiration. This may explain the delay in S4 emergence compared to S2. Early emergence in crops ensures that the stands that were established earlier develop roots that enable them to absorb nutrients and become independent of the food reserve in the propagule (Reed 2022). According to Lawles et al (2012) delayed emergence results in staggard plants on the field which may encourage early weed interference on the field and result in crop failure. The emergence at 6 WAP indicated no significant (P<0.05) variation among the different storage conditions. At 8 WAP, all the treatments achieved 100% emergency. This indicated that the variation in the early stand population was for a short time. At 6 and 8 WAP, the difference in stand count became similar, even though S1 and S3 took the lead.

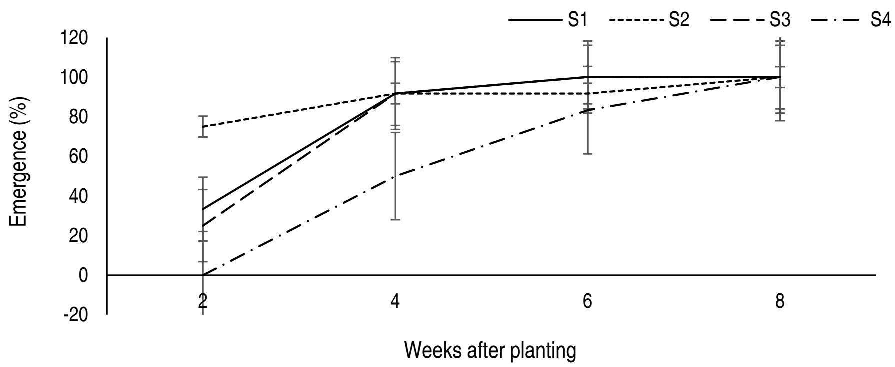

Figure 1. Influence of corm storage methods on the emergence of cocoyam. S1 = Freshly harvested propagule, S2 = propagule stored under shade, S3 = propagule stored in pits, S4 = propagule stored on raised platforms.

Influence of corm storage methods on cocoyam height

The height of cocoyam as influenced by the method of corm storage over 7 months after planting (MAP) is presented in Figure 2. Despite the significant (P<0.05) delay in S4 emergence compared to S2 at 2 WAP and the slow rate to achieve 100% emergence, the plants from S4 were able to close in on S1 and the other treatments concerning height. The height of cocoyam increased progressively for 6 MAP but did not differ significantly among treatments throughout observation periods. However, while S3 and S4 treatments continued to increase in height at 7 MAP, the S1 and S2 heights declined. The increased height for S3 and S4 implied that the plants were still actively growing, while the plants from the other treatments were senescing. The decline in plant height after the maturation of the crop indicates' that the plant is undergoing senescence, except when the decline was as a result of environmental stress (Miryeganeh 2021). The lack of significant difference (*P*<0.05) in height throughout the growth period monitored implies the absence of appreciable variation in the plant's ability to acquire more available resources for development. Similarly, the continuous increase in the S3 and S4 heights suggests that the plants from these two treatments were actively growing, while others were approaching senescence. This could help the plants acquire more resources to increase yield. According to Finch-Savage and Bassel (2016), early attainment in complete emergence will likely increase growth and result in improved yield.

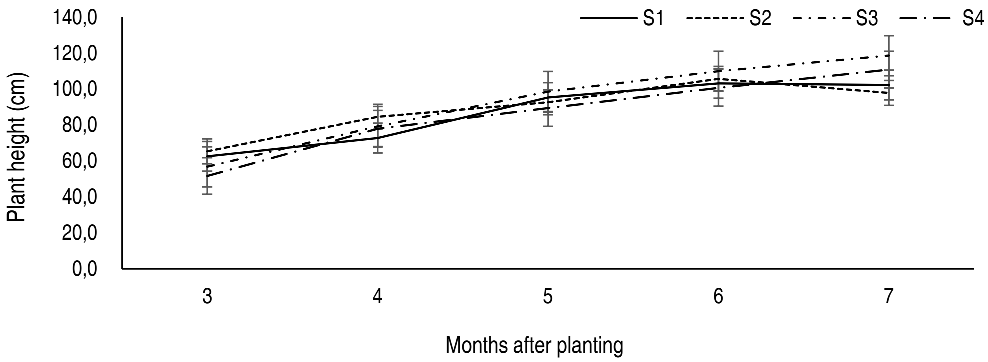

Figure 2. Cocoyam height as affected by corm storage methods. S1 = Freshly harvested propagule, S2 = propagule stored under shade, S3 = propagule stored in pits, S4 = propagule stored on raised platforms.

Cocoyam stem diameter as influenced by corm storage methods

The stem diameter of propagules planted from the different storage conditions did not differ significantly (*P*<0.05) throughout the observation period (Figure 3). Despite the differentials in the time for the plants to emerge, they did not show significant differences (*P*<0.05) in stem diameter during their growth periods. However, S3 consistently had the highest values after the observation made at 3 months after establishment. The plants from S1 had the lowest stem diameter values at 4 and 6 MAP, while S4 had the lowest value at 3 and 5 MAP. The decline in stem diameters for S1 and S2 was at 5 MAP, while the decline was at 6 MAP for plants established from S3 and S4. The fact that S3 and S4 attained the peak stem diameter by a month after S1 and S2 could indicate that the plants could further acquire more nutrients for development and improve crop yield (Miryeganeh 2021). As a consequence, variations in the onset of senescence may lead to differences in harvest times.

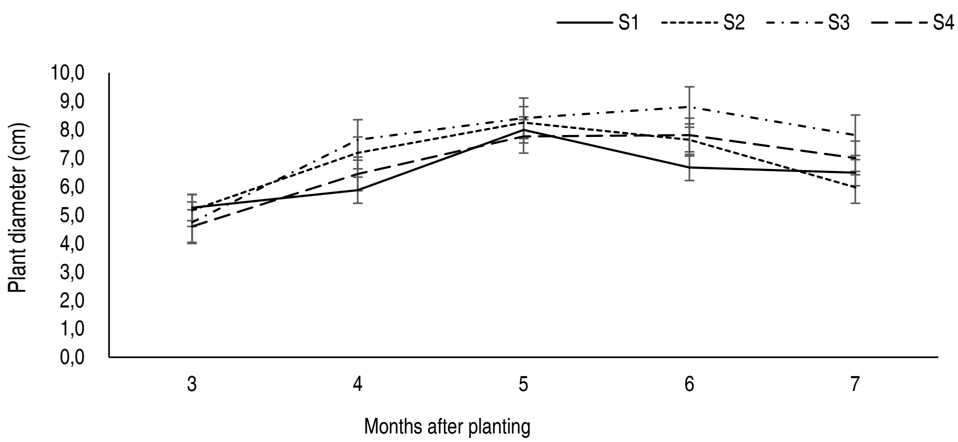

Figure 3. Cocoyam stem diameter as influenced by corm storage methods. S1 = Freshly harvested propagule, S2 = propagule stored under shade, S3 = propagule stored in pits, S4 = propagule stored on raised platforms.

Cocoyam corm yields as affected by propagule storage methods

The corms harvested from the propagule stored under the different conditions did not differ significantly (Figure 4). However, the yield of corms established from the S3 gave the highest value compared to the yields obtained from the other storage conditions. Nonetheless, the corm yield from S3 plant S3 were 15.22, 18.23 and 19.70% higher than the yields from S1, S2 and S4, respectively.

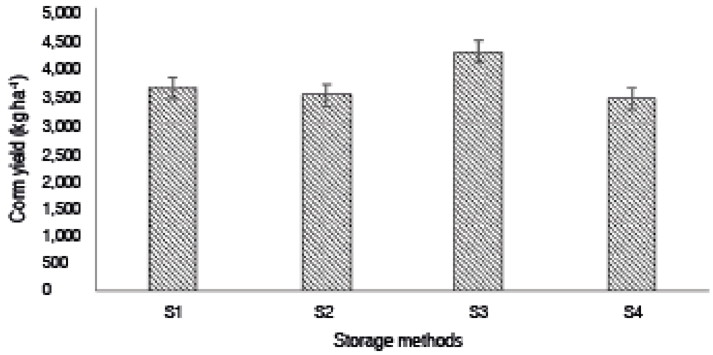

Figure 4. Corm yield as affected by corm storage methods. S1 = Freshly harvested propagule, S2 = propagule stored under shade, S3 = propagule stored in pits, S4 = propagule stored on raised platforms.

Cocoyam cormel yield as influenced by propagule storage methods

The influence of propagule storage conditions on the cormel yield of taro is presented in Figure 5. The highest and lowest cormel yields were observed in the S1 and S4, respectively. However, no significant variation (P<0.05) was observed among the different storage methods. The absence of substantial variation in the yields of cormels under the different storage methods could be attributed to the growth responses observed. Despite the differences in the emergence rate, the propagules from the various methods of storage performed similarly throughout the growth period. Indicating no substantial variation, the ability of the plants to outperform the plant from the other storage conditions. The result was reaffirmed by the non-significant difference (P<0.05) observed in the cormel yield. Nevertheless, the yield from plants propagated through S1 was higher by 858, 754 and 1,275 kg ha^{-1} compared to the observed yield from plants propagated through S2, S3 and S4, respectively. Similarly, a report by Deshi et al. (2021) showed the yield of potatoes stored under different conditions differed from the observed yield after harvest. However, before the freshly harvested corm was collected for subsequent cropping, the sun would have scotched a larger percentage of the corm, thus limiting the available propagule for field establishment.

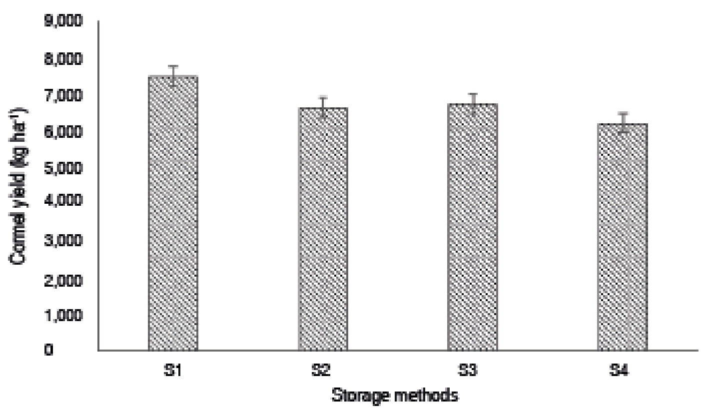

Figure 5. Cocoyam cormel yields as influenced by corm storage methods. S1 = Freshly harvested propagule, S2 = propagule stored under shade, S3 = propagule stored in pits, S4 = propagule stored on raised platforms.

Yield components of cocoyam as affected by the methods of propagule storage

The average length and diameter of corms and cormels produced using propagules from the different storage conditions did not differ significantly (Table 3). However, the propagule from S3 had the highest length and diameter of corms and cormels, while S2 had the lowest values. However, the lack of significant difference in the length and diameter of corms and cormels was not expected due to the absence of appreciable variation in the growth parameters monitored. This response was also reaffirmed by the number of cormels ha^{-1}, with no significant variation among treatments. Nonetheless, S1 differed from S2, S3, and S4 by 3.26, 10.94 and 23.23%, respectively. Although the result was not completely in support of Deshi et al. (2021), inappropriate storage conditions for seed tubers in potatoes could lead to a significant (P<0.05) reduction in crop growth. However, the magnitude of the difference indicated that the variation in growth could be a consequence of improved crop growth under better storage conditions.

Table 3. Yield components of cocoyam as affected by the methods of corm storage.

Storage conditions	Average length corm^{-1}	Average diameter corm^{-1}	Average length corm^{-1}	Average diameter corm^{-1}	Number of cormels ha^{-1}
S1	9.50	7.69	13.00	4.54	82,512.89
S2	8.58	7.65	12.25	4.45	82,485.97
S3	10.33	8.16	13.92	4.64	73,489.83
S4	8.92	7.46	13.42	4.45	63,348.63
LSD	ns	ns	ns	ns	ns

S1 = Freshly harvested propagule, S2 = propagule stored under shade, S3 = propagule stored in pits, S4 = propagule stored on raised platforms.

Correlation coefficient among the observed parameters

The Pearson correlation coefficient indicated a significant (P<0.05) positive relationship between weight loss and storage efficiency (Table 4). However, a significant (P<0.05) negative correlation coefficient was observed between weight loss in storage and corm emergence at 2 and 4 WAP. Although the weight loss also had a negative correlation coefficient with corm weight and number of cormels ha^{-1}, the relationship was not significant (P<0.05). The correlation coefficient indicated a significant (P<0.05) negative relationship between corm emergence at 2 WAP and the weight of cormel produced at harvest. The variation could be due to the lower rate of moisture loss in the taro compared to tannia in storage. Storage efficiency improved with a reduction in the moisture content of the stored produce (Sugri et al. 2017).

Table 4. Pearson's correlation coefficient of the parameters.

	WL	SE	E2	E4	E6	CW	NC
SE	0.55*	-	-	-	-	-	-
E2	-0.53*	0.16	-	-	-	-	-
E4	-0.67*	-0.14	0.59*	-	-	-	-
E6	-0.37	-0.21	0.20	0.72**	-	-	-
CW	-0.01	0.05	-0.28	-0.14	-0.23	-	-
NC	-0.45	-0.17	-0.03	0.35	0.31	0.41	-
WM	0.40	-0.03	-0.69*	-0.36	-0.25	0.37	0.31

*, ** = Correlation is significant at the 0.05 and 0.01 levels of probability, respectively (2-tailed); WL = weight loss; SE = Storage efficiency; E2 = emergence at 2 weeks after planting; E4 = emergence at 4 weeks after planting; E6 = emergence at 6 weeks after planting; CW = weight of corms ha^{-1}; number of corms ha^{-1}; WM = weight of cormels ha^{-1}.

CONCLUSION

The results from the storage conditions study indicated that weight loss and storage efficacy differed among the storage conditions. Weight loss was minimal when storing corms under shade, amounting to 8.95% only. Conversely, storage efficacy was optimal by storing corms on raised platforms (91.20%). Corm emergence was remarkably higher in corms stored under shade and lowest when corms were stored on raised platforms. Propagule storage conditions had no appreciable influence on *Colocasia esculenta* growth and yield. Cormel yields for freshly harvested corms, corms stored under shade, corms stored in pits and on raised platforms were 7,483, 6,625, 6,729 and 6,208 kg ha^{-1}, respectively. In conclusion, storage under shade or in pits proved more suitable for preserving cocoyam corms as propagule in Ayepe and its surrounding environment.

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