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Comparing the yield and the nutritional and nutraceutical composition of pigmented maize landraces (*Zea mays* L.) grown under agroecological and conventional management**

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A b s t r a c t. Maize, a global staple, faces sustainability challenges in conventional farming. This study investigated, in a participatory way, the impact of agroecological versus conventional practices on maize yield and grain composition, focusing on three Mexican landraces of varying colours (yellow, blue, and pink). Measures included grain yield components, bromatological composition, bioactive characterisation, and antioxidant activity to address the concerns of both producers and consumers of maize grains. Agroecological management, incorporating manure, and manual/mechanical pest control yielded 3x more grain than conventional methods. Agroecological grains showed lower fat, protein, and dietary fibre values ($p<0.001$). Bioactives varied significantly ($p\leq0.026$): anthocyanins, carotenoids, flavonoids, and condensed tannins were influenced by variety, while cultivation practices influenced phenolics. Anthocyanin-rich varieties (blue and pink) responded favourably to agroecological management, enhancing soil fertility in high precipitation conditions. Carotenoid-rich maize showed better responses to conventional fertilisation. While agroecological management did not significantly alter grain composition, it outperformed the yield of conventional methods under excess rainfall, increasing nutrient production per unit area. This suggests agroecological approaches could enhance food security for maize producers in the short term and potentially the health benefits of landrace maize consumption, emphasising the importance of sustainable practices for smallholder agriculture and local food systems.

K e y w o r d s: creole maize; agroecological practices; antioxidant activity; phenolics; anthocyanins

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1. INTRODUCTION

Maize is one of the most important crops worldwide, with over 1.2 billion tons of grain harvested in 2023 (FAOSTAT, 2023). Its cultivation area and production have increased steadily over the past 60 years, representing 38% of today's global cereal production, surpassing other crops such as wheat and rice (FAOSTAT 2023). Maize is of great importance for food security, as it provides caloric staples for more than half of the world's population (Guzzon *et al*., 2021; Palacios-Rojas *et al*., 2020). This plant is also relevant for overall human health due to the content of micronutrients like zinc, folate and iron, provitamin A, and vitamin E, as well as phytochemicals or secondary metabolites such as carotenoids and phenolic compounds (Acosta-Estrada *et al*., 2018; Serna-Saldívar *et al*., 2013). These phytochemicals act as antioxidants themselves or interact with enzymes or dietary fibre to enhance body functions and prevent disease (Acosta-Estrada *et al*., 2018).

A great part of the maize production intended for feed, commercial, or industrial uses is associated with current farming systems adopted in countries like the United States, China, or Brazil (Erenstein *et al*., 2022; Liu, 2023). These intensive production systems depend on high-yielding hybrids and improved varieties generated through formal breeding programs that are compatible with monocultures and the use of external inputs like synthetic fertilisers and agrochemicals (Altieri *et al*., 2017; de la Cruz *et al*., 2023). In contrast, most of the maize intended for subsistence farming is produced by smallholder farmers in traditional agroecosystems across many low and middle-income countries (CONABIO, 2017; Ricciardi *et al*., 2018). Such agroecosystems are characterised by low external input utilisation, limited market access and financial solvency, low mechanisation, and limited technology use (CONABIO, 2017). These conditions are suboptimal, in terms of technology and agro-input access and availability, for improved maize varieties or hybrids, which are also costly to acquire given that seeds cannot be maintained season after season. Therefore, subsistence farmers in traditional agroecosystems mostly rely on the use of landraces that are better adapted to the local environment and are compatible with polyculture production and limiting resource contexts (CONABIO, 2017; Guzzon *et al*., 2021). Currently, in Mexico, it is estimated that up to 84% of smallholder maize producers are resource poor and depend on landraces to cover their family needs and, in fact, these farmers contribute around 33% of the country's maize grain supply (INEGI and SADER, 2019; SIAP, 2025).

In the past, government agencies and breeding centres have attempted to increase smallholder adoption of improved or modern hybrid varieties *via* "one-size-fits-all" schemes that promised higher maize yields, offering extension services as well as subsidies to acquire seeds and synthetic inputs (Keleman *et al*., 2009; McLean-Rodríguez *et al*., 2019). Although farmer acceptance of commercial varieties as food staples was mostly unsuccessful (Santillán-Fernández *et al*., 2021), in some regions, it was significant enough to cause the loss of valuable genetic resources (McLean-Rodríguez *et al*., 2019). In many cases, farmers also abandoned traditional practices in favour of adopting herbicide, pesticide, and synthetic fertiliser use (INEGI and SADER, 2019; Santillán-Fernández *et al*., 2021). This situation has brought about environmental issues like the ones found in highly intensified maize production systems, such as water pollution, soil degradation, and pesticide toxicity (Cotler *et al*., 2020; Mardero *et al*., 2018), which, unfortunately, came without generating significant improvements in productivity and poverty reduction for many smallholder producers, particularly in the Centre-South of Mexico (Wies *et al*., 2022; Zepeda Villarreal *et al*., 2020).

Achieving and maintaining the sustainability of maize production is a priority for human development. For this, the rediscovery and conservation of maize landraces and traditional agroecosystems become of crucial importance. On the one hand, traditional maize production systems remain to this day thanks to the farmers' agricultural knowledge that has shaped landraces to their needs and context. One of the reasons for landrace ruggedness is the presence of a high content of secondary metabolites. Studies have shown that, when compared to hybrids or inbred lines, landrace grains are more resistant to oxidative damage, as well as fungal infection and mycotoxin accumulation, due to their more diverse profile of phenolic acids and carotenoids (Chatham *et al*., 2018; Kuhnen *et al*., 2011; Messias *et al*., 2014). Consequently, participatory breeding of landraces could help reduce the use of pesticides and fungicides in modern maize production and contribute to making it a residue-free activity (Landoni *et al*., 2020; Logrieco *et al*., 2021). On the other hand, traditional agricultural knowledge, which is the basis for agroecological management practices, can leverage beneficial interactions between landrace diversity and the associated agrobiodiversity (Altieri and Nicholls, 2017; Palomo-Campesino *et al*., 2021). By doing so, agroecological practices contribute to production through nutrient cycling, soil fertility preservation, pest and disease control, and open pollination (Nicholls and Altieri, 2018). Adopting such practices can help minimise the negative environmental effects of maize agriculture while simultaneously supporting yields in a sustained manner, even in poor seasons (Bezner Kerr *et al*., 2021; Dominguez-Hernandez *et al*., 2020).

Pigmented landraces have garnered increasing interest in recent years due to their nutraceutical potential. Research has investigated the content and profile of phytochemicals such as carotenoids, phenolic compounds, vitamins, and minerals in maize landraces and how transforming maize into different foods could affect these properties and their health benefits (Colombo *et al*., 2021; Domínguez-Hernández *et al*., 2022b). Some studies have also described the effects of agronomic management on yield, morphology, and composition (Giordano *et al*., 2018b, 2018a; Mendoza-Mendoza *et al*., 2019), often using conventional practices. However, the specific effect of low-input agroecological or organic agricultural management on the bioactive profile of maize grains has received limited attention. Some studies have dealt with the effect of different geographical zones on the bioactive profile of landraces cultivated using only organic/agroecological management (Gálvez Ranilla *et al*., 2021; Nankar *et al*., 2016; Uarrota *et al*., 2014). Only one study evaluated the chemical composition parameters in maize landraces cultivated using either conventional or low-input management, however the results were inconclusive (Landoni *et al*., 2021).

With this in mind, a participatory study was set to investigate the effects of agroecological and conventional cultivation practices on the proximal and nutraceutical composition of maize grains and how they affected some yield parameters in three Mexican landraces. The ultimate goal was to determine if adopting a system based on agroecological practices could offer tangible benefits to both the producer and consumer of maize grains in addition to their recognised environmental advantages.

2. MATERIALS AND METHODS

2.1. Plant material and cultivation practices

Three maize landraces of the *Conico* racial group were studied in this work, all originated in the municipality of Ahuazotepec, located in the Sierra Norte of the state of Puebla, Mexico. The varieties are known by their local names: Blackish blue Elotero azul/negro, Pink-purple Xucuyul, and Yellow Amarillo Chico (Fig. 1). They are adapted to grow in the high-altitude valleys prevalent in the area, approximately at 2 800 m above sea level, and in the same edaphoclimatic conditions.

Fig. 1. Typical grains and cobs of three landrace varieties from the Cónico racial group evaluated in 2020 and 2021. From left to right: Elotero azul, Xucuyul, and Amarillo Chico.

Maize cultivation in the 2020 production cycle was done by three farmers, following an overall management that was identified as part of a transition system that combines both agroecological and conventional practices (Dominguez-Hernandez *et al*., 2018), and it was considered a "baseline" for the experiment. Planting in 2020 was done in late April and harvesting was done in mid-October when the plants reached harvest maturity (black layer). Cob samples of each variety were collected from this harvest (baseline/transition year samples), dried in the shade (13-15% moisture content), and stored by the researchers for laboratory analyses. Mass selection criteria were used to select seeds of the different varieties to be used in the 2021 cycle from the 2020 harvest.

In 2021, the harvested seeds were provided for cultivation to cooperating farmers that followed either agroecological or conventional practices in their own rainfed fields in a participatory study. The participating producers owned fields in communities of Ahuazotepec, with an average size of 1.08 (± 0.27) ha, with soils classified as medium textured Andosols. Seeds were allocated in such a way that each landrace was cultivated in 2021 by at least three conventional and three agroecological producers (three replicates/farmers per method). To facilitate monitoring by researchers but not interfere with farmer activities, experimental plots of 0.1 ha were delimited within each participating field. For that cycle, seeds were planted or sown on May 15th (±2 days). All fields were mechanically ploughed prior to sowing, and seeds were planted at a density of 62 500 plants per hectare using 4-row planters. In the agroecological farmer group, weed control was performed using either machine or animal traction, approximately 20 days after sowing (das). Pest management in this group was done by each farmer according to their personal knowledge and criteria; different methods were used: biological insecticide or repellent solutions, diatomaceous earth applications, traps, manual collection, etcetera. In the conventional farmer group, herbicide was applied by the farmers at 20das, using approximately 2 L ha^{-1} Dicamba+Atrazine (Marvel™). Fertilisation was the only cultivation parameter prescribed to the cooperating farmers during the study to standardise nutrient inputs. Soil analyses were not conducted before sowing, since such analyses are not always feasible for smallholder farmers. Fertiliser levels were those used in the municipality, usually applied at planting, and based on recommendations from extension workers and/or agro-chemical sales representatives. Organic fertiliser applied by the agroecological group was composted ovine manure at a dose of 7.5 Mg ha^{-1} and was spread manually in three stages: planting (50%), 30das (25%), and 60das (25%). The chemical fertilisation dose (120N-60P-30K) used in the conventional fields was applied as follows: P and K were applied at 20das, and the N application was split at 40 and 60das. Both fertilisers (organic and chemical) were applied ensuring even distribution across the furrow and near the radicular zone. No irrigation was provided during the cycle. Harvest took place 6 months after sowing, between November 15th and December 2nd, when the plants reached harvest maturity. At each cooperating farmer field, researchers selected two central rows of 10 m each within the experimental plot, where ears were sampled for laboratory analyses and then dried in the shade at room temperature to an approximate moisture content of at least 14% to be later stored until use. Rainfall and temperature data were monitored and recorded daily during 2020 and 2021 using a Vantage VUE station (Davis Instruments, Hayward, USA). The monthly averages of these parameters for the production cycle and historical data are also reported.

2.2. Grain yield components

Grain yields (GY) for each cycle, management, and variety were self-reported by the farmers once they finished harvesting. For 2021, the results presented are the average of three replicates for each production method (conventional or agroecological). The other measured yield components were hectolitre or test weight (HW, method 14-40; AACC, 1976) and 100 grain weight (100 GW), all done in triplicate.

2.3. Laboratory analyses

2.3.1. Sample preparation

A total of 20 cobs per variety, production method, and cycle were obtained from each cooperating producer. The ears were shelled manually, and the grains were cleaned from any debris or foreign material. Prior to analyses, the grains were ground in a spice mill (80350R, Hamilton Beach, USA) and sieved through a 0.5 mm screen. All grain samples and flours were stored in plastic bags at 4°C.

2.3.2. Bromatological analyses

Standard AOAC methods were used to determine the contents of lipid (920.39), ash (942.05), protein (920.15), and insoluble and soluble dietary fibre (991.43) (AOAC, 2002). Total non-fibre carbohydrate contents were obtained by difference. These analyses were performed in triplicates. Moisture was measured using a thermobalance apparatus (RS232, Newtry, China) by drying the samples at 120°C until weight loss was less than 1 mg per minute.

2.4. Bioactive quantification and antioxidant activity

2.4.1. Extraction of bioactive compounds

Phenolic extracts were made adapting the microscale method proposed by Zavala-López and García-Lara (2017) in triplicates of 50 mg ground samples of the field-grown landraces. Briefly, 80% methanol was used to extract free phenolics after incubation (15 min at 25°C, 500 rpm) and centrifugation (5000 rpm, 10 min; D1008e, DLAB Scientific, China). The pellet was subjected to alkaline hydrolysis (90°C, 500 rpm) in a thermomixer (DBS100C, Joanlab Eq., China) for 1h and later acidified. Three washes of ethyl-acetate were used to recover bound phenolics after centrifugation. These extracts were combined, evaporated to dryness, and resuspended in 1mL of 50% methanol. All the extracts were stored at −20°C until their analysis. Free and bound reconstituted extracts were filtered through a nylon syringe filter (0.45 µm pore size, Pall Life Sciences, USA) and stored in black tubes at -20°C until use.

For anthocyanin extraction, 20 mg triplicates of ground pink and black samples were extracted with acidified methanol (1% trifluoroacetic acid) at 4°C under 150 rpm agitation for 75 min, followed by centrifugation at 14000 rpm for 5 min. The extraction procedure was repeated twice, and the extracts were kept separate. In the case of carotenoids, maize grains from all three varieties (200 mg) were finely ground and suspended in 2 mL of water-saturated butanol in black tubes. The tubes were vortexed for 45 s, shaken at low speed for 15 min, and left to stand for 60 min at room temperature in the dark. These processes were performed thrice. Finally, the supernatants were recovered and centrifuged at 4000 g for 10 min.

2.4.2. Total contents of bioactive compounds

Appropriate dilutions of the bound and free methanolic extracts were prepared to measure total contents of phenolics (TPC), flavonoids (TFC), and condensed tannins (TCT). TPC were assayed via a reaction with 10% Folin-Ciocalteu reagent, neutralisation with 7.5% sodium carbonate, and 90 min incubation. The results were reported as mg of ferulic acid equivalents (FAE) per 100 g (dry weight basis, DW). TFC were quantified through a reaction with a 1% solution of 2-aminoethyldiphenylborinate and expressed as mg of rutin equivalents (RE) per 100g $_{DW}$. TCT were based on the reaction with acidified vanillin (1% vanillin and 4% HCl in methanol) and reported as mg (+) catechin equivalents (CE) per 100g $_{DW}$. All the above absorbance measurements were performed in a UV-Vis microplate spectrophotometer (Multiskan GO 1510, Thermo-Fisher Scientific, USA).

In the case of total anthocyanin contents (TAC), the absorbance of each of the obtained extracts was measured at 520 nm against a pelargonidin chloride standard curve. The results of the two extracts were combined and reported as mg of pelargonidin equivalents (PE) per 100g $_{DW}$. For total carotenoid content (TCC), the absorbance of the supernatants was read at 450 nm. TCC was quantified using a lutein standard curve and thus they were expressed as mg of lutein equivalents per kg $_{DW}$. For TAC and TCC measurements, an UV/V spectrophotometer (VE-5100UV, VELAB, Mexico) and semi-micro plastic cuvettes were used.

2.4.3. Antioxidant activity

Total antioxidant activities of the methanolic extracts were determined in the free and bound extracts of the landraces using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard curves. The TEAC-ABTS [TEAC, Trolox Equivalent Antioxidant Capacity; ABTS, 2,2-azinobis-(3-ethylbenzothazoline-6-sulphonic acid)] and the FRAP (Ferric Reducing Antioxidant Power) assays were performed as reported by (Loarca-Piña *et al*., 2019) and absorbance was measured at 734 and 593 nm, respectively. The oxygen radical absorbance capacity (ORAC) assay was used to obtain the hydrophilic antioxidant capacity in extracts diluted 1:75 following the method reported previously (Domínguez-Hernández *et al*., 2023).

2.5. Statistical treatment

The results of the proximate composition, grain yield parameters, and phytochemical contents of the maize samples were analysed using ANOVA for a randomised block design, where effects were considered significant at $p<0.05$. For significant treatments, a Tukey test was used to compare means with a significance level of 5%. The treatments were the combinations of two factors: Variety (black Elotero, yellow Chico, or pink Xucuyul) and Agronomic management (Conventional or Agroecological). Additionally, the samples of these treatments were compared to the baseline (samples obtained from the transition year 2020). Pearson correlations were also performed among the nutraceutical parameters. A principal components analysis (PCA) considering the agronomic, nutritional and nutraceutical parameters to find the variables that better described the varieties and management used to grow the grains in 2021. Samples from the baseline or transition year were not used for this since they combined practices from the two managements. Clustering using Euclidean distances (Ward's hierarchical method) was then created to highlight any differences between the samples cultivated and the parameters measured in this work. All procedures were done using Minitab 19 (Minitab Inc, USA).

3. RESULTS AND DISCUSSION

3.1. Agronomic parameters

The average weather conditions during 2020 and 2021 for the municipality, along with historical data, are shown in Fig. 2. Maximum temperature decreased between the cropping seasons (April to December) from 15.7°C in 2020 to 14.2°C in 2021. The behaviour relates to historical data from 1985-2010, with a mean maximum temperature of 14.9°C. Cumulative cycle rainfall in 2020 and 2021 was 717.7 and 1035.1 mm, respectively, while historical data recorded an average of 975.5 mm. In 2020, hail and frost events were recorded at the end of August and early October, respectively. The increase in precipitation during the second year spanned through the whole cycle, with heavy rains starting in May, reaching a peak of 229.2 mm in August, and then continuing throughout most of September and October (Fig. 2). In this period, reduction in rainfall happened in July, with 20mm less than the same month in 2020. Historical precipitation patterns show a continuous rainy season starting in May, starting an increase in August and reaching a peak in September, close to the one observed in 2020.

Table 1 shows the yield and main physical characteristics of the harvested grains of three maize varieties grown under different agronomic managements and over two cropping seasons. HW and 100 GW were significantly affected by management and maize variety ($p \leq 0.050$). Yellow Chico tended to have higher HW than the other two varieties, but the differences were only significant for HW. For 100 GW, the yellow grains tended to have smaller weights than the black Elotero ones. In terms of grain sizes, measured as HW and 100 GW, they were, respectively, 5.6 and 8.8% higher in the baseline year. These differences were only significant for the HW of the Elotero and Xucuyul varieties, and for the 100 GW of the black and yellow maize.

The changes in yield components during 2021 were likely caused by adverse weather conditions. In this year, rains were first delayed, which affected the sowing date, but once they started, they were abundant, which caused water-saturation in the soil, leading to seedling death and the need to resow some of the plots. The excess rainfall in 2021 was particularly severe during the stages of pollination and grain filling (as can be seen in Fig. 2), which accounted for the decrease in grain size (Tian *et al*., 2019). Grain yield was also affected during the 2021 season in both agroecologically and conventionally grown maize ($p \leq 0.001$), but no differences between the varieties were observed ($p = 0.800$).

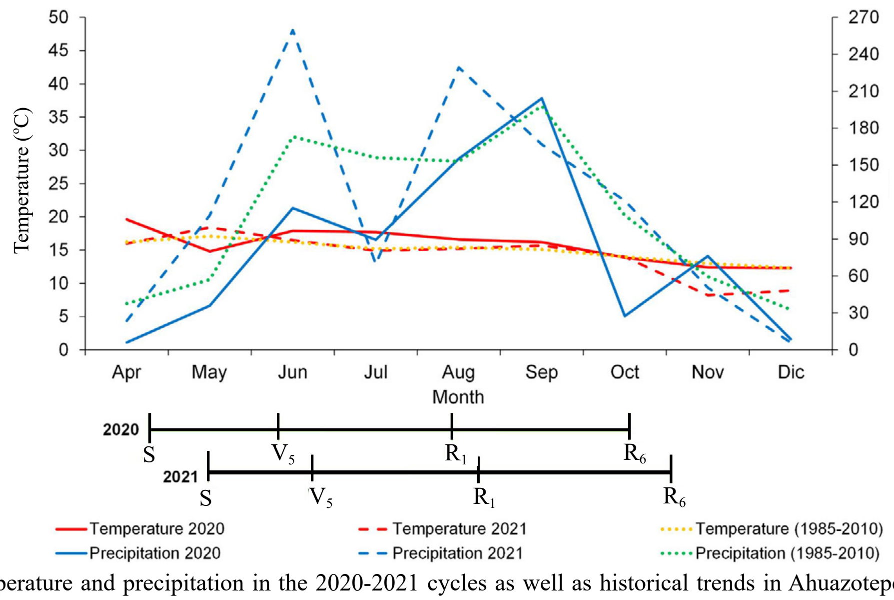

Fig. 2. Maximum temperature and precipitation in the 2020-2021 cycles as well as historical trends in Ahuazotepec, Mexico. The phenological stages of maize for each cycle are also depicted: S – sowing, V_5 – fifth ligulated leaf, R_1 – flowering, R_6 – physiological maturity.

Table 1. Chemical composition and selected yield components of landrace maize varieties produced using different practices: agroecological and conventional

Variety	Moisture	Ash	Fat	Protein	Carbohydrates		Hectolitre weight (kg hL^{-1})	100-grain weight (g)	Grain yield (Mg ha^{-1})
					Total dietary fibre	Available			
	($g\ kg^{-1}$)								
Baseline (Transition 2020)									
Black Elotero	15.0±7.07^{a}	18±0.12^{ab}	55±0.04^{bc}	88±1.26^{f}	212±0.07^{a}	627±0.08^{d}	70.8±1.29^{a}	35.2±0.09^{a}	4.5±1.37^{a}
Pink Xucuyul	13.1±4.45^{b}	16±0.09^{c}	55±0.01^{bc}	102±1.20^{c}	189±0.10^{ab}	638±0.12^{bcd}	67.1±0.29^{b}	33.8±0.80^{ab}	3.6±0.72^{abc}
Yellow Chico	12.9±3.25^{b}	18±0.10^{ab}	59±0.01^{b}	98±1.20^{d}	181±0.03^{ab}	645±0.01^{abc}	74.2±0.38^{a}	32.8±0.23^{bc}	3.6±1.08^{abc}
Agroecological (2021)									
Black Elotero	13.4±2.40^{b}	18±0.14^{ab}	43±0.01^{d}	91±0.10^{e}	182±0.07^{ab}	666±0.10^{ab}	66.1±0.30^{bc}	30.6±0.39^{d}	4.2±0.42^{ab}
Pink Xucuyul	13.3±1.62^{b}	19±0.05^{a}	42±0.01^{d}	98±1.40^{d}	202±0.11^{ab}	639±0.04^{bcd}	63.1±0.35^{c}	31.9±0.18^{bcd}	5.0±0.26^{a}
Yellow Chico	11.4±1.63^{d}	18±0.08^{a}	49±0.01^{c}	110±1.20^{b}	171±0.16^{b}	652±0.15^{ab}	73.5±0.11^{a}	30.4±0.58^{d}	6.1±1.80^{a}
Conventional (2021)									
Black Elotero	12.0±4.24^{c}	12±2.01^{d}	40±0.05^{d}	98±0.10^{d}	203±0.03^{a}	648±0.04^{abc}	64.6±0.80^{bc}	31.7±0.69^{cd}	1.7±0.53^{bc}
Pink Xucuyul	11.7±0.49^{cd}	16±0.23^{bc}	42±0.02^{d}	91±0.10^{e}	194±0.10^{ab}	658±0.10^{a}	62.8±0.26^{c}	30.8±0.41^{cd}	1.6±0.46^{bc}
Yellow Chico	11.3±4.10^{d}	18±0.09^{a}	67±0.01^{a}	118±1.20^{a}	179±0.03^{ab}	618±0.04^{cd}	71.6±0.65^{a}	31.6±1.62^{cd}	1.4±0.43^{bc}

Ash, fat, protein, and carbohydrates are expressed in terms of dry matter. Different letters indicate statistical difference between the means (Tukey test, $p<0.050$). Values are reported as mean ± standard error. Seeds were cultivated in 2020 using a combination of agroecological and conventional practices.

In general, the average yield of these varieties (3.5 Mg ha^{-1}) was well within the range of moderate productivity reported for maize landraces (Giordano *et al*., 2018b; Mendoza-Mendoza *et al*., 2019; Nankar *et al*., 2016). In the region of study, it has been reported that some producers were able to obtain yields above 4 Mg ha^{-1} while using practices consistent with agroecological or transition methods (Dominguez-Hernandez *et al*., 2018). On average, the yield produced under agroecological cultivation was 1.3 times that of the baseline/transition year and over 3 times higher than that of maize produced under conventional management (Table 1). Previous reports also showed that manure application produced an average yield of almost 7 Mg ha^{-1} using the black Elotero landrace, which was 1.4 Mg ha^{-1} more than under chemical fertilisation (Domínguez-Hernández *et al*., 2022a). In our study, however, we speculate that this effect, rather than indicating an increase in yield under agroecological cultivation, points towards a loss of yield in the conventional method due to excessive rainfall. This phenomenon has been associated with yield decreases due to lixiviation of nutrients, as well as reduced uptake of nutrients and hypoxia, which are known to affect plant development (Kaur *et al*., 2017). These negative effects are more common in systems that rely only on synthetic fertilisers, as their use is associated with depletion of organic matter, which leads to decreases in soil water infiltration and holding capacity as well as soil fertility (Matta and Reeves, 2020; Pimentel *et al*., 2005). Additionally, application of pesticides in conventional agriculture contributed to the disruption of microbial, radicular, and invertebrate activities that are necessary for the formation, accumulation, and stabilisation of soil organic matter (Matta and Reeves, 2020). Agroecological production, in contrast, stimulates biological activity and incorporates organic residues, maintaining soil health and soil organic matter levels (Dhaliwal *et al*., 2019; Matta and Reeves, 2020). These effects likely helped to protect and sustain the moderate yields of landrace maize produced under agroecological cultivation.

3.2. Nutritional composition of the grains

The proximal composition was affected by both management and maize variety ($p\leq0.011$). Chico maize showed the highest contents of protein, ash, and fat, followed by

Xuxuyul and Elotero maize. In contrast, dietary fibre and available carbohydrates were highest in the latter varieties than in the yellow one. The overall composition of this Cónico racial group was within the ranges reported for landraces grown in the Mexican highlands (Flint-Garcia *et al*., 2009; Vázquez-Carrillo *et al*., 2011). In our study, the higher contents of available carbohydrates in the flours of anthocyanin-pigmented varieties could relate to softer grains. This could be seen in their HW, which is an indirect measure of grain hardness (Salinas Moreno *et al*., 2013), and was effectively lower than that of yellow maize (Table 1). Traditionally, the differences in hardness have determined the use local people give to each variety: black Elotero and pink Xucuyul are the first choices for nixtamalisation, wet-milling, and traditional tortilla-making, while the harder yellow Chico is primarily used as feed and secondly to supplement tortilla production as the availability of stored softer grains wanes.

Ash contents increased or were maintained in all three varieties when grown agroecologically, but other changes depended more on the variety. Fat and protein increased in yellow maize, but they decreased in pink maize. Available carbohydrates, on the other hand, were maintained or increased their levels in the seeds of all three varieties and both crop growing treatments. Total dietary fibre showed no significant variation with respect to the baseline/transition year values in all three varieties (Table 1). The results indicate that genotype influenced the grain composition to a greater degree than the treatment. Landoni *et al*. (2021) also observed that the cultivation method did not influence pigmented maize grain composition to a greater extent than genotype. In their study, however, ash, starch, oil, and lignin were higher in low-input than in the conventional system. Similarly, other studies have found increases in the levels of sugar/carbohydrates and protein/amino acids of maize, wheat, and oats when produced under organic and/or low-input fertilisation (Chauhan *et al*., 2020; Omondi *et al*., 2022; Thakur *et al*., 2021). It was expected that applications of organic fertiliser would increase the availability and utilisation of soil nutrients, as well as provide the plants with micronutrients like Zn or S, all of which could have influenced mineral accumulation, as well as the biosynthesis of starch, fat, protein, and fibre (Dhaliwal *et al*., 2019; Thakur *et al*., 2021). In the present study, the use of manure only increased ash contents, having overall lower fat, protein, and fibre values than conventionally grown grains ($p<0.001$).

This suggests that split chemical fertilisation could have prevented additional N losses caused by excess water in such a way that the synthesis of biomolecules was not impaired in the plants (Kaur *et al*., 2017), even when the crop failure was high and thus the grain yields decreased. The agroecological management did not improve the overall grain composition but protected grain production, resulting in greater production of nutrients per unit of cultivated area, which could represent a greater benefit for the food security of maize producers. In general, our findings agree with previous observations that grain composition trends may not necessarily mirror yields since the governing mechanisms are often diverse and more complex (Wang *et al*., 2020). Additionally, Fisher *et al*. (2020) observed that the benefits of high-quality organic fertilisers on the levels of grain nutrients are often diluted in medium to high quality soils, such as the ones that are prevalent in our study site.

3.3. Nutraceutical parameters

3.3.1. Contents of phytochemicals

The free and bound contents of the different bioactive components can be seen in Fig. 3 for all three varieties and cultivation treatments. Both management and variety affected these quantities significantly ($p\leq0.026$). However, it was the latter factor that accounted for most of the variability observed in anthocyanins, carotenoids, flavonoids, and condensed tannins, while for phenolics it was the cultivation method. Black Elotero contained 67% more TAC than pink Xucuyul and, although the contents in both varieties were generally higher under agroecological cultivation, no significant changes with respect to cultivation were observed (Fig. 3a). The overall TAC obtained in this study coincide with results for other blue and purplish landraces such as Rojo ancho, Rojo criollo, Cónico norteño, Bolita, and Olotillo azul (López-Martínez *et al*., 2012; Mendoza-Díaz *et al*., 2012; Salinas-Moreno *et al*., 2012). Carotenoids increased in 2021 when compared to the baseline and tended to be higher in the agroecological samples (Fig. 3e). These compounds were present in all three varieties regardless of the cultivation method. The highest concentration was found in agroecological maize with 34.8 mg_{LE} per kg_{DW}. On average, TCC in Chico were 1.6 and 4.4 times larger than those of Elotero and Xucuyul, respectively. These results are consistent with the TCC range observed in white (<10 mg kg_{DW}^{-1}), creamy-white (≤22 mg kg_{DW}^{-1}), and yellow (≤30 mg kg_{DW}^{-1}) endosperm varieties around the world (Domínguez-Hernández *et al*., 2022b), colours that correspond to the endosperm of our pink, blue, and yellow landraces, respectively. However, in the case of blue and purple pigmentations, carotenoids in the endosperm are often masked by the colour of the outer layers, even though they may be present in measurable levels (Kuhnen *et al*., 2011).

Regarding TFC, no clear trends or significant changes were observed with regards to cultivation (Fig. 3c). Flavonoids in the samples studied were mainly found as part of the free fraction (from 46 to 62% of the total). Free flavonoid contents were higher in black maize, while the bound fraction was higher in the pink variety. When compared to the darker varieties, yellow maize had intermediate levels of free flavonoids, but overall lower levels of bound ones. Similar trends were found in (Suriano *et al*.,

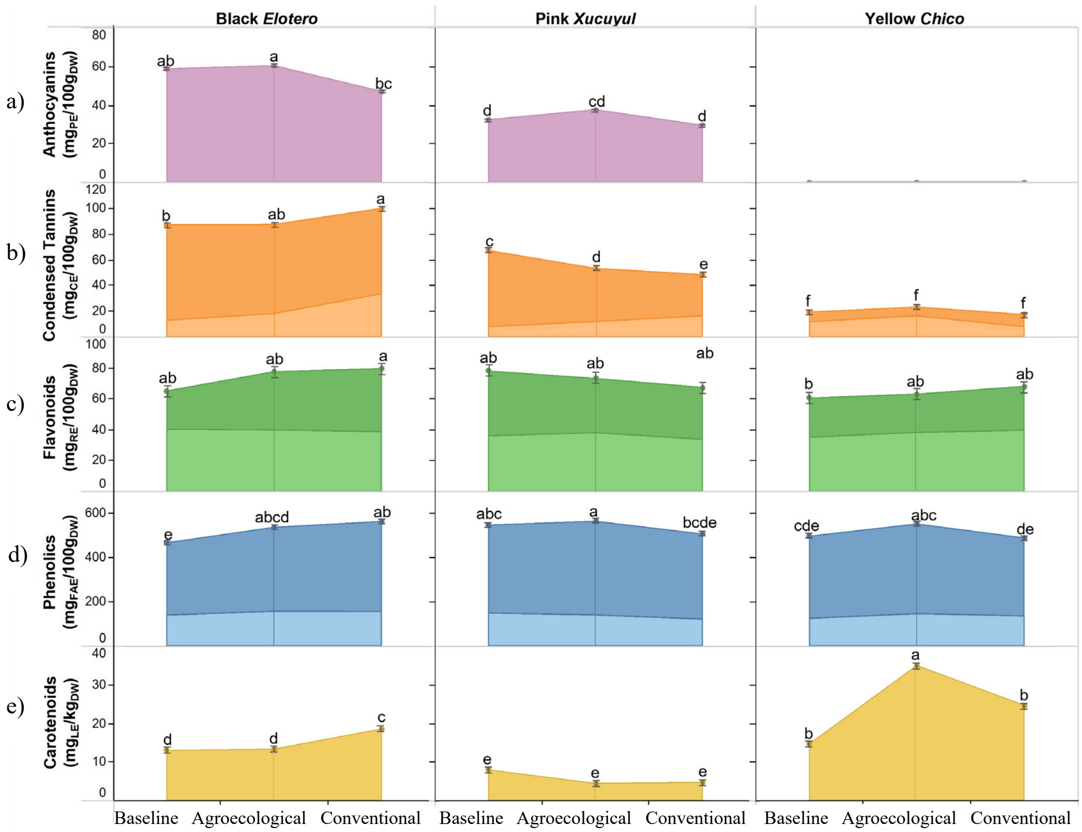

Fig. 3. Total contents of bioactive compounds: a) anthocyanin, b) condensed, c) flavonoids, d) phenolics, e) carotenoids; in landrace maize cultivated using different management approaches, agroecological, conventional as well as transition (baseline, combining agroecological and conventional). Light shade: free fraction. Dark shade: bound fraction. Results are expressed as mean ± standard deviation. Letters indicate differences between the means. Means not sharing letters are significantly different (Tukey test, $p < 0.050$).

2021) for total flavonoid levels in Italian yellow, blue, and red maize. The values of free and bound TCT, also known as proanthocyanidins, can be seen in Fig. 3b. Overall, yellow maize had significantly lower TCT, as well as the lowest proportion of bound tannins (between 30 and 55%), while this percentage in black and pink maize was above 70%. According to (Rodríguez-Salinas *et al*., 2020) and (Herrera-Sotero *et al*., 2017), proanthocyanidins constitute an important part of flavonoids in Mexican landraces of blue, purple, and red maize, but not in yellow one. This discrepancy with the moderate TCT levels in our Chico variety could be because the vanillin assay is rather unspecific and often quantifies monomeric flavonols, in addition to proanthocyanidins (Schofield *et al*., 2001). For total phenolic contents, shown in Fig. 3d, on average, Xucuyul had higher TPC than Chico, while Elotero contained intermediate levels. Although bound phenolics were present as the major fraction (avg. 73% of TPC) in all three varieties, pink and yellow maize contained their larger proportion than black grains (Fig. 3d). The values of TPC are in the range of previous observations of landraces with similar pigmentation (Domínguez-Hernández *et al*., 2022b).

Agricultural management practices with their impact on nutraceutical components have been previously investigated in some maize landraces; however, it is difficult to establish the magnitude of their effect, as it cannot be separated from other factors, such as soil type, nutrient levels, and their interaction with such aspects as rainfall levels and temperature (Giordano *et al*., 2018a; Uarrota *et al*., 2014). In European landraces, low-input management promoted the synthesis and accumulation of anthocyanins in blue *Millo Corvo* and carotenoids in orange-red *Scagliolo* (Landoni *et al*., 2021). In terms of fertilisation, for yellow, orange, and red Italian landraces under high rainfall, cell-bound phenolic acids, and carotenoids were found to increase with applications of nitrogen fertiliser (Giordano *et al*., 2018b). In Mexican blue and red varieties, anthocyanins and TPC but not TFC were enhanced by high levels of organic matter, phosphorus, and other micronutrients (Martínez-Martínez *et al*., 2019). In our study, phenolics, tannins, flavonoids, and anthocyanins in agroecological Elotero and Xucuyul maize tended to be statistically equal or higher than those from conventional management. Similarly, the responses of yellow Chico indicated that the conventional cultivation failed to increase phenolics and carotenoids to the same degree as the agroecological treatment. In contrast, carotenoids in the anthocyanin-pigmented landraces seemed to respond better to conventional practices, although the effect was only significant for black Elotero.

In general, the results agree with previous recommendations with respect to planting maize in conditions that ensure sufficient macro and micronutrient availability for adequate synthesis of different maize metabolites and to improve nutraceutical value (D'Amato *et al*., 2019; Giordano *et al*., 2018b). In the varieties that were studied here, it seems that maintaining soil quality and fertility and organic matter provision (an indicator of soil carbon) are equally important, hence the benefits of agroecological fertilisation and pest management. An increase in bioactives, significant or otherwise, would also be expected under this cultivation, mainly because the plants were exposed to additional biotic stresses since agrochemicals are not used (Landoni *et al*., 2021). However, a recent study suggested that pest resistance could also be mediated by soil (Mutyambai *et al*., 2019). In the aforementioned research, soil conditioning to agroecological practices (push-pull, polyculture) increased secondary metabolism and growth rate of maize, and although the exact mechanisms were not elucidated, the resistance was attributed to an improved ecosystem, in terms of organic matter content, nutrient availability, and accompanying micro and macro-organisms.

The response of carotenoids in the present study indicated that higher rainfall and lower temperatures, as observed in 2021, enhanced their synthesis in the medium and high containing maize varieties, but not in the low-containing one (Giordano *et al*., 2018b). The literature indicates that maize carotenoids are affected by similar factors than yield, increasing with available nitrogen fertiliser and longer maturation periods (Giordano *et al*., 2018a, 2018b; Landoni *et al*., 2021) but not with other nutrients such as initial phosphorus fertilisation (Lux *et al*., 2021). However, the effects are highly dependent on the studied variety, and it seems that low-input management could increase carotenoids and yield only in yellow Chico. This, paired with the contrasting trends observed in Elotero and Xucuyul, suggested that carotenoids exhibit a larger dependence on the interaction of environmental factors and genotype than that of phenolics when *e.g.* nitrogen is not the limiting nutrient. However, additional tests are needed to confirm this assertion.

The overall stability of TFC, considering the trends of TPC, TAC, and TCT, seems to indicate that the plants carry out a fine-tuned phenolic and flavonoid synthesis to respond to seasonal stressors. To find the possible relationships between the different bioactive groups, a correlation analysis was performed for each variety, which can be seen in Fig. 4. According to the results, in black Elotero, increasing levels of condensed tannins correlated with lower anthocyanin contents ($r=-0.600$, $p=0.047$) and higher phenolics ($r\geq0.650$, $p\leq0.039$, Fig. 4). For pink Xucuyul, phenolics and flavonoids were each correlated to anthocyanins and tannins, respectively ($r\geq0.814$, $p\leq0.007$). In the case of yellow Chico, moderate to strong significant correlations were found between tannins and phenolics ($r\geq0.531$, $p\leq0.042$). The differences in the strength and direction of these correlations also indicate that the response is highly dependent on the variety, even in similar conditions. Variability with respect to specific phenolics in response to environmental challenges has been observed in maize and other grains, such as wheat and rice (Chen *et al*., 2020; Dixon and Sarnala, 2020; Khang *et al*., 2016). In general, enhanced levels of phenolics, and particularly flavonoids, in different plants have been associated with biotic and abiotic pressures caused by higher precipitation, such as fungal or bacterial disease, herbivore attack, or preharvest sprouting (Khlestkina, 2013). Regarding water-saturated or waterlogged soils, the upregulation of phenolic synthesis and accumulation corresponded with increased tolerance in *e.g.* onions, wheat, potato, and rice (Chen *et al*., 2020; Dubey *et al*., 2020; Khang *et al*., 2016; Orsák *et al*., 2020). Specifically regarding proanthocyanidins in the studied pigmented maize, greater accumulation in plant tissues has been found to occur also due to leaching and decreasing availability of N, as part of the plant's tolerance and response to improve nutrient use efficiency (Narvekar and Tharayil, 2021; Yu *et al*., 2020). Carotenoids also play a part in the response to oxidative stresses, and although they

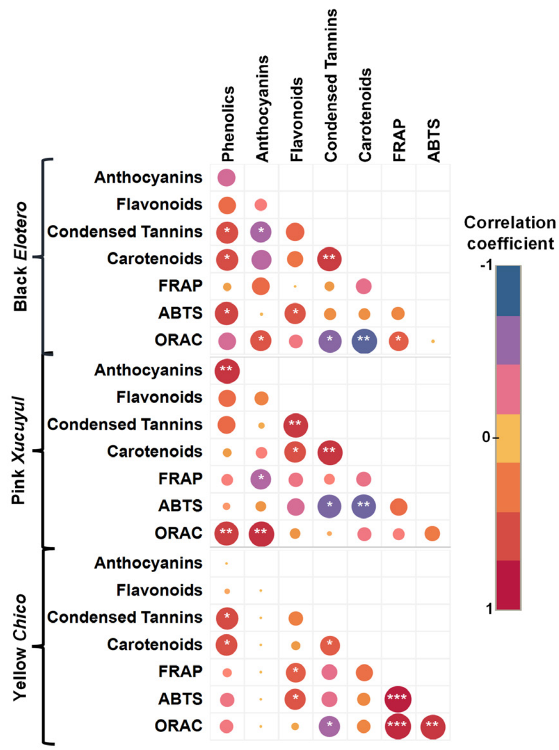

Fig. 4. Correlation matrices between bioactive contents and antioxidant capacity of three maize landrace varieties. Significance of the correlations is indicated as follows: $*p\leq0.050$; $**p\leq0.010$; $***p\leq0.001$.

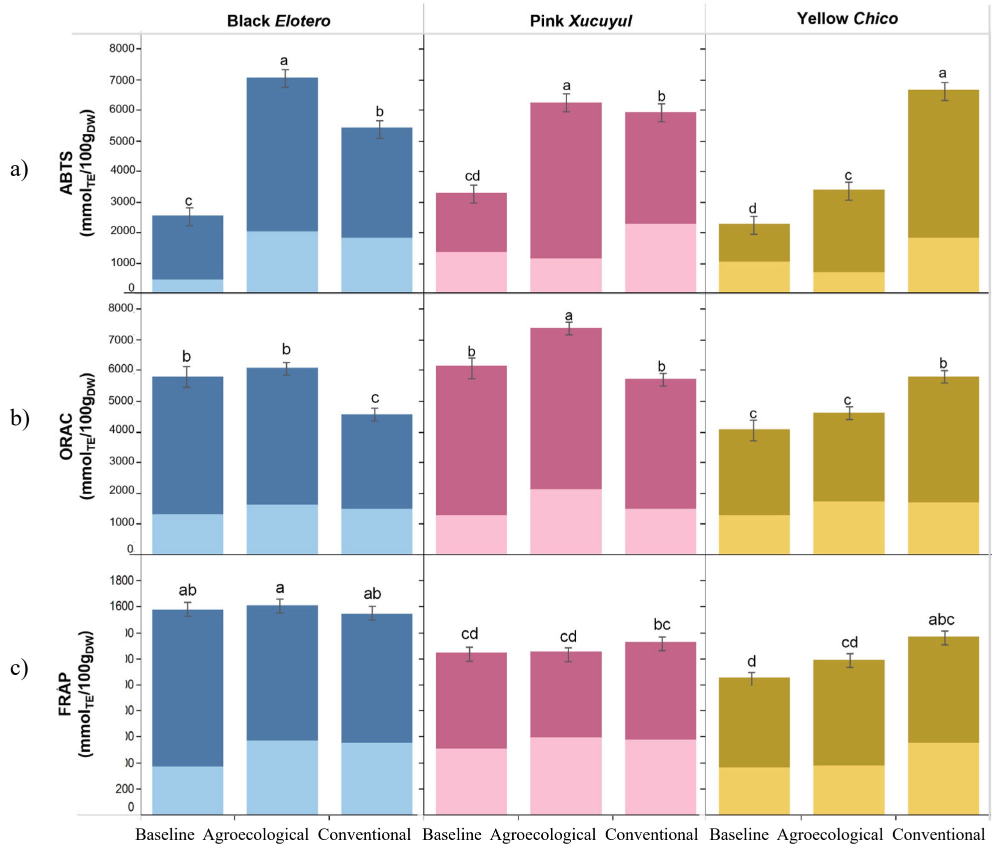

Fig. 5. Total antioxidant activity in landrace maize cultivated using different management approaches, agroecological, conventional as well as transition (baseline, combining agroecological and conventional). Light columns: free fraction. Dark columns: bound fraction. Results are expressed as mean ± standard error of the mean. Letters indicate differences between the means. Means that do not share a letter are significantly different (Tukey test, $p < 0.050$): a) ABTS, b) ORAC, c) FRAP.

have been shown to decrease in waterlogged conditions in some cereals, the change is smaller in tolerant varieties (Pais *et al*., 2023). For the studied maize landraces, although carotenoid levels were better explained by genotype and environmental conditions, it could be expected they increased or were maintained along other antioxidant species that indicated tolerance to excess moisture levels. Indeed, in our study, carotenoids were significantly correlated with condensed tannins in all three varieties and with phenolics and/or flavonoids in one or two of them (Fig. 4).

3.3.2. Antioxidant activity

The three measures of free and bound antioxidant activity used in this study are shown in Fig. 5. The combined effects of management and maize variety were significant for ORAC, FRAP, and ABTS ($p \leq 0.040$). The contribution of variety to the observed variability was higher in ORAC and FRAP, while for ABTS it was management. For all three measurements, bound compounds provided most of the antioxidant activity of the studied maize varieties, ranging from 63% (FRAP) to 72% (ORAC). This is generally the case for raw maize, since phenolic acids, contributing to antioxidant activity, are mostly found linked to the cell walls of the grains. In terms of the varieties, Elotero and Xucuyul maize presented, on average, higher antioxidant activities than Chico ($p \leq 0.001$). One reason for this could be that the contribution of such components as anthocyanins was absent in the latter variety. However, given the sizes of the differences in the antioxidant assays (ABTS: 1.22-1.26x, FRAP: 1.06-1.31x and ORAC: 1.12-1.33x), other compounds, such as flavonols and coextracted carotenoids, may have contributed to the antioxidant response in the yellow variety. When comparing 2020 and 2021 values, FRAP showed little variation, while ABTS and ORAC generally increased in the second year (Fig. 5.). In general, the behaviour observed in antioxidant activity can be attributed to the nature of the assays and their sensitivity and affinity to the compounds present in the extracts, thus the varieties showed different or complementary results. In black and pink maize, ABTS was, respectively, 2.4 and 1.8 times higher than in the other seed, but larger differences were seen under agroecological management (Fig. 5a). ORAC also tended to increase with the application of the practices specified above, significantly in the case of pink Xucuyul (Fig. 5b). For both ORAC and ABTS, the use of conventional practices generally produced lower activity in anthocyanin-pigmented landraces. In the case of FRAP, a lack of change was evident for most varieties, especially pink maize (Fig. 5c). For yellow Chico and black Elotero, however, a ~50% increase in free FRAP was obtained during 2021 (Fig. 5c). In all three antioxidant measures, conventional management produced significant increases in yellow maize (3 times in ABTS, 1.47 in ORAC, and 1.3 in FRAP) with respect to baseline levels.

As with the levels of bioactives, the increases in antioxidant activity observed during 2021 may have been a consequence of the accumulation of water (ponding, flooding, and/or waterlogging) under high precipitation. According to Tian *et al*. (2019), water-saturated soil conditions cause an increase in reactive oxygen species, such as superoxide anion $O_2^{\cdot-}$ and hydrogen peroxide (H_2O_2), leading to injuries and senescence in maize hybrids. Tolerance to these conditions is provided by upregulation of enzymatic and non-enzymatic antioxidant systems, the latter being largely associated with radical scavengers like phenolic compounds (Alhdad *et al*., 2013; Chen *et al*., 2020; Dubey *et al*., 2020; Khang *et al*., 2016; Orsák *et al*., 2020). Ren *et al*. (2017) studied onion cultivation under different managements (organic *vs*. conventional) and environmental conditions (yearly rainfall variation causing waterlogging), finding that increases in phenolics and flavonoids were positively correlated to *in vitro* antioxidant activity measures (FRAP and DPPH). To test if our samples showed similar behaviours, our correlation analysis also included relationships between bioactive parameters and the different antioxidant activity measures (Fig. 4). Elotero's ABTS was directly related to phenolic and flavonoid contents ($r \geq 0.650$, $p = 0.042$), while ORAC was positively correlated with anthocyanins ($r \geq 0.610$, $p = 0.049$) and negatively with condensed tannins ($r = -0.714$, $p \leq 0.031$). For Xucuyul, ORAC increased with higher TAC and TPC ($0.781 \leq r \leq 0.870$, $p \leq 0.013$, Fig. 4). In the case of Chico, moderate to strong correlations were found between phenolics and all three antioxidant measures ($0.582 \leq r \leq 0.764$, $p \leq 0.007$), as well as between flavonoids and ABTS ($r = 0.566$, $p = 0.018$). Additionally, in pink Xucuyul, anthocyanins and tannins had strong negative correlations with FRAP ($r = -0.589$, $p \leq 0.034$) and ABTS ($r = -0.761$, $p \leq 0.017$), respectively. Although no correlations were significant, the relationships between carotenoids and antioxidant activities differed in direction according to the variety, being positive for yellow maize but negative for black and pink (Fig. 4). These last observations do not imply a negative or non-existent contribution of Xucuyul's anthocyanins or carotenoids to antioxidant activity. Regarding pink maize, the results could be due to differences in the specific anthocyanin profile of black maize; for example, studies have found dark blue maize can contain more active forms, such as cyanidin derivatives, than pink or red varieties (Peniche-Pavía and Tiessen, 2020; Sytar *et al*., 2018), which could enhance the response. On the other hand, given the hydrophilic nature of the activity assays, correlations with TCC would not be necessarily expected (Domínguez-Hernández *et al*., 2022). However, the presence of carotenoids as coextracted antioxidant species cannot be discounted in naturally rich landraces like yellow Chico. According to Hwang *et al*. (2016), some polar carotenoids like xanthophylls can be extracted in the aqueous phase of the extraction solvent providing additional antioxidant effects measurable by hydrophilic assays.

Taken together, our results indicate that upregulation of antioxidant activity due to secondary metabolites was present in grains cultivated in 2021, which could suggest a degree of inherent tolerance to excess moisture stress in the studied landraces. Additionally, the overall changes in ORAC and ABTS in anthocyanin-rich varieties with respect to agroecological cultivation mirrored the effect of additional biological stressors in the absence of herbicides and pesticides and improved soil health due to the effect of organic fertilisation on the synthesis of secondary metabolites. The behaviour of yellow maize differed from the other varieties due to the presence of carotenoids, which responded positively to conventional management. However, to fully understand and describe their contribution to the stress response in high precipitation conditions, additional antioxidant measures are required.

3.4. Agroecological and conventional fertilisation: Cluster analysis

The variables used for clustering were selected from the first and second principal components, which accounted for 64.8% of the total variability in the samples cultivated in 2021. The chosen variables were TAC, TCT, ABTS, ORAC, TCC, Dry matter, Fat, Protein, Ash, Carbohydrates, HW, and Yield, with absolute weights of 0.35, 0.32, 0.38, 0.34, 0.28, 0.31, 0.20, 0.47, 0.32, and 0.27, respectively.

Using these items in the PCs, the cluster tree was constructed using the Euclidean distance. The starting clusters were each of the 2021 observations, which combined in distinct hierarchical groups as the distance between them increased. The resulting dendrogram plot in Fig. 6b revealed that the maize samples could be clustered in two groups, one containing Elotero and Xucuyul and a second one containing Chico. This could be expected and indicates that the main source of variation or distance between the clustering items is the primary pigment in the grains: either anthocyanins or carotenoids, as can also be seen in the Biplot (Fig. 6a). In addition to the pigment, Fig. 6a shows the two groups of landraces could be differentiated by variables related to their nutritional composition (Chico) or those describing their bioactivity (Elotero and Xucuyul).

However, the two types of management also showed increasing distances within the samples/observations of the same pigmentation. Agroecologically grown yellow maize was branched apart from conventionally grown one; similarly, black and pink varieties were clustered in a group

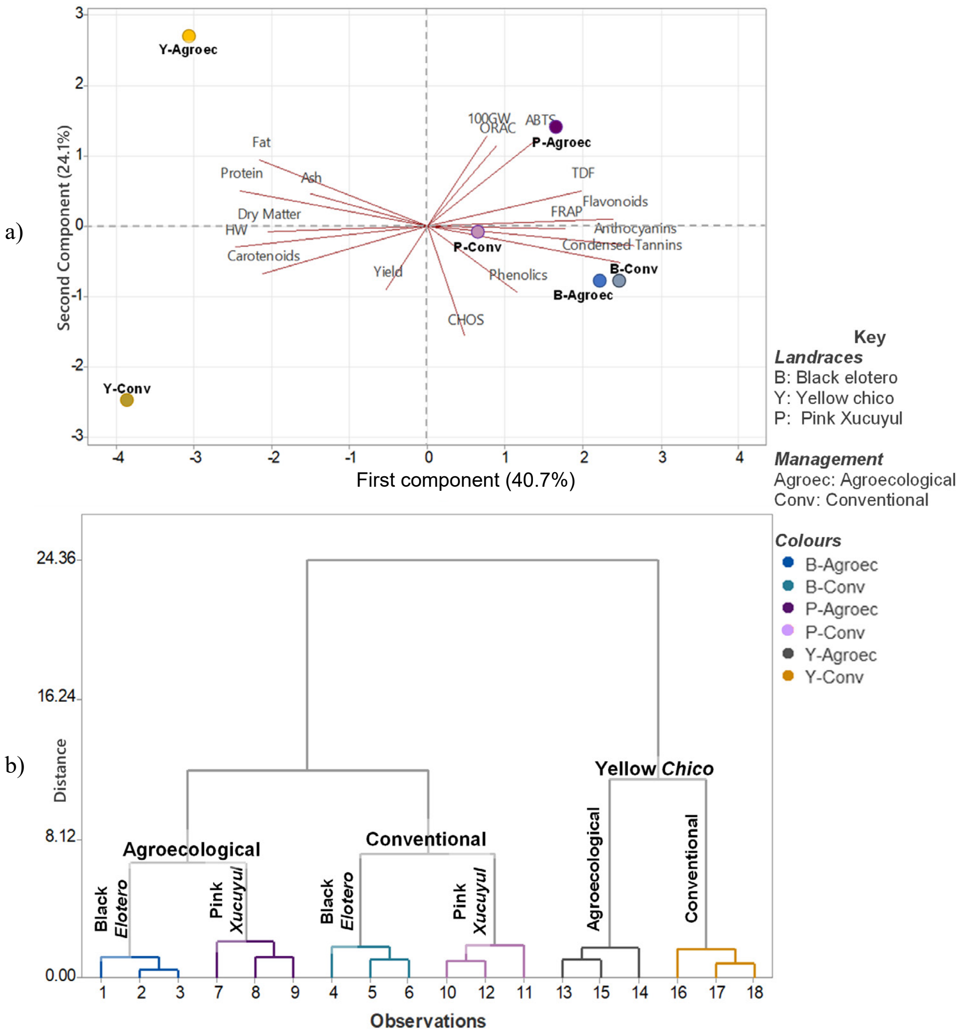

Fig. 6. Cluster analysis of the maize samples obtained in 2021 using agroecological or conventional management: a) principal components analysis biplot of yield, nutritional and nutraceutical grain parameters; b) dendogram (Euclidean distances, Ward's hierarchical method) showing the grouping of the different sample observations.

separated from conventional samples, although the differences were not as marked as with yellow maize (Fig. 6a and b). Indeed, as can be seen in the PCA biplot, anthocyanin-rich varieties appear to be clustered together more closely than yellow ones (Fig. 6a). Our results are in accordance with a recent study on Italian landraces, showing that is possible to separate samples of agroecological and conventionally grown maize landraces in terms of their yield, nutritional and/or nutraceutical properties (Landoni *et al.*, 2021). Although the study design and the environmental conditions prevalent in our study are not sufficient to generalise the findings presented without additional trials, other studies have also been able to successfully differentiate crop species that are grown using sustainable agronomic practices (Nascimento *et al.*, 2020; Vaitkeviciene *et al.*, 2020).

It is worth noting that, even though there may be differences between the two managements, there is no scientific consensus on whether organic or agroecological cultivation produces better foods than conventional practices (Cruz-Carrión *et al.*, 2023; Popa *et al.*, 2019). Nevertheless, consumption of organic/agroecologically grown foods may be desirable from a health and environmental perspective, given the lack of pesticide use and the preservation of soil health that comes with such practices. In this context, the generation and dissemination of information related to the separate nature of agroecological maize landraces could be advantageous when marketing to conscious consumers. For our cooperating farmers, this could encourage adoption of agroecological practices by those in the conventional group as a means for adding value to their production in the short term. There is also the economic advantage of not using inputs that are volatile in price and add to the production costs. In the long term, opening marketing opportunities, reducing costs, and sustaining yields would aid in the conservation of local landrace varieties.

4. CONCLUSIONS

The application of agroecological practices in the cultivation of three pigmented maize landraces (*Zea mays* L.) demonstrated significant advantages in terms of yield and resilience in excess rainfall conditions, compared to conventional management. Agroecological management, which incorporated organic fertilisation and manual/mechanical pest control, resulted in grain yields that were higher than those achieved under conventional practices. This highlights the potential of agroecological approaches to enhance food security for smallholder farmers, particularly in regions prone to unpredictable weather patterns and extreme climatic events.

In terms of nutritional composition, the results indicated that genotype and environmental conditions had a greater influence on grain composition than the cultivation method. However, the agroecological practices showed a tendency to improve soil health and nutrient retention, which may have contributed to the stability of grain yields in adverse conditions. The anthocyanin-rich varieties (blue and pink) responded favourably to agroecological management, with enhanced soil fertility and organic matter content playing a key role in their performance. In contrast, the carotenoid-rich yellow variety showed better responses to conventional fertilisation, suggesting that nutrient availability and management practices should be tailored to the specific phytochemical profiles of different landraces.

The nutraceutical analysis revealed that agroecological practices positively influenced the accumulation of bioactive compounds, particularly phenolics and anthocyanins, in the anthocyanin-rich varieties. This suggests that agroecological management not only supports yield stability but may also enhance the health-promoting properties of maize grains. The observed upregulation of antioxidant activity in agroecological samples further underscores the potential of these practices to improve the nutritional quality of maize, particularly in stress conditions such as excess rainfall.

Future research should focus on long-term studies to assess the effects of transitioning towards agroecological practices, investigate soil health and nutrient dynamics, and explore detailed phytochemical profiles of maize landraces under different management systems. Additionally, given the increasing frequency of extreme weather events, studies should evaluate the climate resilience of landraces to stressors like drought and flooding. Finally, they could integrate traditional agricultural knowledge with modern agroecological practices in a participatory manner to co-design and evaluate management strategies that further align with their needs and contexts.

Conflicts of interest: The authors declare that there are no conflicts of interest.

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