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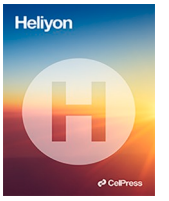

Characterization of the antioxidant activity, carotenoid profile by HPLC-MS of exotic colombian fruits (goldenberry and purple passion fruit) and optimization of antioxidant activity of this fruit blend

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ABSTRACT

The consumption of antioxidants can prevent chronic non-communicable diseases and the exotic Colombian fruits, goldenberry (Physalis peruviana L.) and purple passion fruit (Passiflora edulis f. Edulis Sims), are rich in bioactive compounds. The aim of this work was to characterize and optimize the antioxidant activity of these fruits blend. The fruits were classified according to their maturity stages, the freeze-dried extracts were physiochemically characterized, and polyphenols, carotenoids and antioxidant activity were quantified, and an experimental mixture design was applied to optimize the antioxidant activity of the bend. For the goldenberry the maturity stage 3 had higher iron-reducing capacity and higher content of polyphenols. Meanwhile, for the purple passion fruit, this maturity stage had higher antioxidant activity by all methodologies and a higher concentration of polyphenols; the ultrasound-assisted extraction showed statistical differences for polyphenols, ABTS and FRAP. Antioxidant activity showed significant differences ($p < 0.05$) between samples (TBARS (3.98 ± 0.14 and 7.03 ± 0.85 μM-MDA/g), ABTS (36.53 ± 2.66 and 29.4 ± 4.88 μMTrolox/g), DPPH (36.53 ± 2.66 and 23.90 ± 0.96μMTrolox/g), ORAC (23.02 ± 0.36 and 32.44 ± 0.94 μM Trolox/g) and total polyphenols (5, 29 ± 0.34 and 9.12 ± 0.37mgGA/g). Some of the carotenoids identified by HPLC-MS in both fruits were lutein, α and β-carotene, phytoene and lycopene. The optimum bend was goldenberry 0.83 and purple passion fruit 0.17.

1. Introduction

Chronic non-communicable diseases (NCDs) are the leading cause of death in the world [1]. Furthermore, the current COVID-19

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pandemic shows a trend of premature deaths in low- and middle-income countries such as Colombia, given the strong impact of COVID on people who suffer from NCD., proving again the importance of preventing NCDs as cofactors of morbidity and mortality from infectious diseases [2]. NCDs share pathophysiological mechanisms such as inflammation and oxidative stress [3]. For this reason, there is a public and scientific interest in seeking therapeutic and prophylactic strategies that improve these disorders. High-impact interventions are those with an emphasis on prevention with modifiable risk factors such as diet [4]. Healthy dietary patterns can help to prevent NCDs, especially antioxidant-rich foods, as fruits and fruit juices [5]. Research has shown that adequate consumption of fruits and vegetables exhibits multiple health benefits, closely related to their high content of bioactive compounds such as vitamins, carotenoid, and polyphenolic compounds [6]; and there is a relationship between increased fruit intake and the prevention of some NCDs. For example, Lai et al., 2015 [7], evaluated the relationship between total fruit intake and mortality from cardiovascular diseases (CVD) in women. They found that mortality from CVD occurred in older women with higher BMI, lower intake of vitamin and mineral supplements and lower physical activity. Furthermore, participants with an intake of more than 7 servings of fruit per day had a 43% lower risk of death from CVD compared to women who consumed less than 2.5 servings of fruit per day. In another study, other studies showed that the consumption of fruit berry juice can increase the antioxidant capacity of the blood serum and lower the level of triglycerides [8], significantly reduce systolic blood pressure, as well as total cholesterol [9]. Finally, Tian et al., 2021 [10] studied the antioxidant and anti-inflammatory activity of the polyphenols, luteolin, kaempferol, apigenin, and quercetin, separately. They found that luteolin, kaempferol, and quercetin have an antioxidant activity greater than that of BHT (commercial antioxidant); and luteolin and quercetin showed better activity than vitamin C. The United Nations General Assembly designated 2021 as the International Year of Fruits and Vegetables (IYFV), holding that most people do not eat enough fruits and vegetables. This initiative complements the Sustainable Development Goals that deal with nutrition, consumption, and health [11]. Exotic Colombian fruits are in high demand in international markets. They are desired for their quality, flavor, and health benefits. Goldenberry (Physalis peruviana L.) and purple passion fruit (Passiflora edulis f. Edulis Sims) are some of the fruits with greater international commercial importance. For 2019, Colombia exported 4545 tons with a value of 20.18 million dollars (USD FOB) [12].

For the purple passion fruit in 2020, the value of exports increased 3.8%, from 33.25 in 2019 to 34.51 million dollars (USD FOB) [13]. These fruits are rich in bioactive compounds (BC); the goldenberry is native from the South American Andes and is an important source of provitamin A (α-carotene, β-carotene, and β-cryptoxanthin), vitamin C, E, K1, and vitamin B complex [14]; and some polyphenolic and carotenoid compounds, such as rutin, myricetin, quercetin, kaempferol, β-carotene, α-carotene, and lutein, have been fully identified in this fruit [15]. Some medicinal properties have been reported for goldenberry: antioxidant, anti-inflammatory, antitumor, hypocholesterolemic, antidiabetic, diuretic, antiseptic, sedative, and analgesic [16–19]. On the other hand, the genus Passiflora is native from tropical areas in South America. Colombia is the country with the greatest diversity of the genus Passiflora with 170 species. In general, these fruits are rich in minerals and vitamins, especially A and C, thiamin, riboflavin, and niacin. They are also a good source of carotenoids, anthocyanins, and alkaloids [20], as well as riboflavina and niacina [21]. The purple passion fruit has triterpenoids, flavonoids, glycosides (passiflorine), cyanogenic glycosides, aromatic compounds, flavonoid glycosides (luteolin-6-*C*-chinovoside), alkaloids and saponins; the purple passion fruit also has carotenoids such as β-carotene, vitamins such as L-ascorbic acid (vitamin C), vitamin A, riboflavin, resveratrol, piceatannol, choline, g-lactones, esters, volatile oils, eugenol, amino acids, and a large number of polyphenolic compounds, flavonoids and anthocyanins [22,23]. Although it is less studied than other Passiflora, it has antioxidant, anti-inflammatory, anti-tumor, and anti-diabetic medicinal properties [24,25]. The bioaccessibility of these compounds is crucial for them to have biological activity in humans, However, the absorption of polyphenols varies greatly, with reported bioaccessibility ranging from 0.3% to 43%, and metabolite concentrations in the plasma are usually much lower. On the other hand, carotenoids and other fat-soluble compounds require digestive enzymes to release fatty acids and monoacylglycerol to form micelles for absorption through the water barrier and into intestinal enterocytes [26].

Combinations of BC may exhibit additive, synergistic, or antagonistic interactions due to their structure diversity and may have complementary antioxidant activities. Approaches from a single antioxidant are not adequate to assess health benefits, given that BC from natural sources are always mixtures. The pharmaceutical industry has paid much attention to the antagonistic interactions between nutrients and drugs; however, food-food interactions have not received the same interest. Although both synergistic and antagonistic interactions between foods are important, particularly for functional foods that contain high levels of BC, further interactions between food components are also important and depend on many factors, including in vitro activities, food processing, and human metabolism [27]. Some food industry segments are developing differentiated products composed of two or more foods like fruit. This is done with the purpose of elaborating exclusive and healthier foods because they can enhance the nutritional characteristics like the content of vitamins, minerals or BC to the product, thereby adding value to the final product [28]. The aim of this study was to identify the antioxidant activity of exotic Colombian fruits (goldenberry and purple passion fruit) according to its maturity status, characterize carotenoids profile by HPLC-MS, and optimize the antioxidant activity of this fruit blend.

2. Materials and methods

2.1. Materials

Exotic Colombian fruits were selected according to the Colombian technical standards for fresh fruits NTC 4580 [29] and NTC 6456 [30] for goldenberry and purple passion fruit, respectively. For this study, category two fruits were purchased in the food market of Rionegro and La Ceja, Antioquia. These category two fruits are not suitable for export and are consumed in local markets.

The fruits were classified according to ripeness, following the color parameters described in the NTC 4580 [29] and NTC 6456 [30] for goldenberry and purple passion fruits respectively. The maturity index was calculated with the titratable acidity, which was carried

out by potentiometric titration with 0.1 M NaOH, and the °Brix were quantified at 20 °C in a refractometer, according to the Colombian technical standard NTC 4624 [31].

Approximately 100 g of each fruit were washed, disinfected by immersion into sodium hypochlorite at 100 ppm for 20 min, and rinsed. For the extraction process, the gulupa was pulped manually given its physical characteristics while the goldenberry was subjected to extraction whole and without a basket, their edible part were blanched in water at 78 °C for 5 min in order to inactivate enzymes, such as polyphenoloxidase and peroxidase, which catalyze the degradation process of the BC under shearing or crushing [32, 33], which, in the presence of oxygen, transform the polyphenols of fruits into benzoquinones [34].

2.1.1. Purple passion fruit extraction

The blanched fruits were processed in a cold press extractor at 60 rpm at room temperature, and water at 60 °C was added in ratio 1:5 to the residues, for a second extraction. The two extracted phases were mixed and adjusted to 7 °Brix (in order to avoid caramelization during the freeze-drying process). The freeze-dried extract was stored in the dark at −20 °C until its final use.

2.1.2. Goldenberry extraction

The blanched fruits were processed in a cold press extractor at 60 rpm at room temperature, and water at 60 °C (in 1:5 ratio) was added to the residues for a second extraction assisted by ultrasound, according to the methodology described by Civan and Kumcuoglu et al., 2019 [35], with some modifications. Using an ultrasound bath with an amplitude of 60% for 5 min, the extraction was made at 60 rpm; the two extracted phases were mixed and adjusted to 7°Brix. The freeze-dried extract was stored in the dark at −20 °C until its final use.

2.2. Characterization of fruit extracts

The freeze-dried extracts were characterized physiochemically according to the official methodologies by the AOAC (protein, fat, ash, and moisture) [36]. Additionally, 200 mg of these extracts were reconstituted with 5 mL of water, and the BC and antioxidant activity were quantified.

2.2.1. Total polyphenols content

Total polyphenolic content was determined by spectrophotometry according to Nunes et al., 2018 [37], with minor modifications. Briefly, 30 μL of the sample was mixed with 150 μL of Folin-Ciocalteu reagent 0.2 N (1:10) and 120 μL of Na2CO3 (7.5%). The mixture was incubated at 45 °C for 15 min, protected from light, and for 30 min more at room temperature. Finally, absorbance was measured at 765 nm in a UV–Vis Multiskan spectrometer (Thermo Scientific). The content of total polyphenols was determined by means of a standard curve of gallic acid 0–110 μM/mL. All measurements were carried out five times.

2.2.2. Total carotenoids content

Total carotenoid content was determined by spectrophotometry according to Biswas et al., 2011 [38] and Sánchez-Camargo et al., 2019 [39] with minor modifications. 100 mg of the sample were mixed with 5 mL of acetone at 4 °C, vortexed for 1 min and allowed to set for 30 min at 4 °C, vortexed again for 5 min, and centrifuged at 1370 g for 10 min. This procedure was repeated 3 times, the supernatants were mixed, and absorbance was measured at 450 nm in a UV–Vis Multiskan spectrometer (Thermo Scientific). The total carotenoid content was determined by means of a standard curve β-carotene (32 μg β-carotene/mL −0.031 μg β-carotene/mL). All measurements were carried out three times.

2.3. Characterization of the antioxidant activity for goldenberry and purple passion fruit extracts

2.3.1. DPPH• (2, 2-diphenyl-1-picrylhydrazyl radical) scavenging ability

In 96-well microplates, 100 μL of sample and 100 μL of DPPH reagent in methanol at 200 μM were mixed and incubated in a dark at room temperature for 30 min. The absorbance was measured in a Multiskan UV–Visspectrometer (Thermo Scientific) at 550 nm. The antioxidant activity was determined using a standard Trolox curve 0–100 μM ($r^2 = 0{,}9974$) [37,40,41]. All measurements were carried out five times.

2.3.2. Ferric reducing antioxidant power (FRAP)

In 96-well microplates, 35 μL of sample and 265 μL of FRAP reagent (buffer 0.3 M: TPTZ 10 mM: Ferric chloride 20 mM (10:1:1)) were mixed, incubated in the dark at 37 °C for 30 min. Absorbance was measured in a Multiskan UV–Vis spectrometer (Thermo Scientific) at 595 nm. The antioxidant activity was determined by means of a standard Trolox curve 0–500 μM ($r^2 = 1$) [37]. All measurements were carried out five times.

2.3.3. ABTS + anion radical scavenging activity

In 96-well microplates, 10 μL of sample and 190 μL of ABTS + radical (ABTS 14 mM - Potassium Persulfate 4.8 mM (1:1) in distilled water at absorbance 0.7 ± 0.02 a 740 nm) were mixed. The mixture was left to react in the dark at room temperature for 6 min, and the absorbance was measured in a Multiskan UV–Vis spectrometer (Thermo Scientific) at 740 nm. The antioxidant activity was determined by using a standard Trolox curve 0–250 μM ($r^2 = 0{,}9943$) [42]. All measurements were carried out five times.

2.3.4. (TBARS) inhibition of lipid peroxidation of buffered egg yolk by extracts

For this, 200 μL of sample, 1 mL of an emulsion of egg yolk in phosphate buffer 0.1 M pH 7.4 (25 g/L) and 100 μL iron sulphate Fe2+ 1 mM were mixed and left at 37 °C for 1 h. Subsequently, 1 mL of trichloroacetic acid at 15% (w/v) and 0.5 mL of thiobarbituric acid 0.02 M were added and incubated at 90 °C for 45 min, the tubes were cooled on ice bath and centrifuged at 4440 g for 10 min, and the absorbance of supernatant was read at a wavelength of 532 nm [43–46]. The antioxidant activity was determined by means of a standard malondialdehyde curve (MDA) (0–100 μM). All measurements were carried out three times.

2.4. Carotenoid profile by HPLC-MS

Fruit extract (0.5–1.5 g) and 5 mL of miliQ water were mixed. The carotenoids were extracted with 20 mL of hexane/acetone/ethanol (50:25:25, v/v/v) and BHT 0.1%, vortexed for 1 min, and centrifuged at 35,000 g for 5 min. This procedure was repeated twice. The organic portion was dried in a speed vac and re-suspended in 4 mL of methanol/methyl *tert*-butyl ether (50:50, v/v) with 0.1% BHT, dried again with speed vac, it was volumetrically resuspended in 2 mL methanol/methyl *tert*-butyl ether (50:50, v/v), filtered through a 0.22 μm membrane and finally injected in the HPLC-MS equipment [15].

An Accucore C30 column (2.6 μm, 150 × 2.1 mm) (Thermo Fisher Scientific) was used in two mobile phases. Phase A was methanol/methyl tert-butyl-ether/water (85/5/10, v/v/v) enriched with 5 mmol/L of ammonium formate, and Phase B was methanol/methyl *tert*-butyl ether/water (11/85/4, v/v/v) enriched with 5 mmol/L of ammonium formate, The methodology and gradient program were developed following the protocol outlined by Etzbach L et al., 2018 [15].

2.5. Experimental design and statistical analysis

The results were expressed as mean ± standard deviation (SD). Comparisons of the means were made with the Fisher LSD test with a 95% confidence level. The Simplex lattice mixture design with two components, goldenberry (0–1) and purple passion fruit (0–1) and five response variables, antioxidant activity by: ABTS, DPPH, FRAP, TBARS, and ORAC as described in the previous paragraphs, was applied using STATGRAPGICS 19 ® with the purpose of evaluating the effects and optimizing proportions in the mixes. Nine mixes were formulated with different proportions described in Table 1.

3. Results and discussion

3.1. Effect of maturity stage on the antioxidant activity

The antioxidant activity of both fruits was measured using different methods and showed statistically significant differences between the mean of each evaluated variable and the state of maturity, with a 95% statistical confidence.

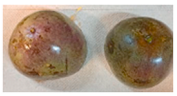
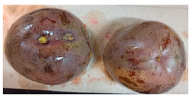
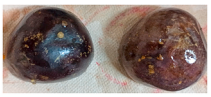
The results of the state of maturity according to the color tables of the Colombian technical standards for fresh fruits NTC 4580 [29] and NTC 6456 [30] for goldenberry and purple passion fruit, respectively, are described in Table 2. The change of color in many fruits is the most noticeable characteristic during their maturation. The degradation of the green color is associated with the synthesis of pigments whose colors range from yellow to red-purple that are linked to the presence of carotenoids and some anthocyanins [47]. The goldenberry's states of maturity were 1, 3, 5, and 6, with a maturity [29] index of 4.33 ± 0.04, 7.84 ± 0.14, 8.90 ± 1.06, and 10.00 ± 0.38, respectively, which coincide with those reported in NTC 4580. For the purple passion fruit, the states of maturity were 3, 4, and 6; and the maturity indexes 3.21 ± 0.45, 4.49 ± 0.50, and 5.38 ± 0.36, respectively, which coincide with those reported for Pinzón et al., 2007 [47].

The antioxidant activity was quantified by the ABTS, FRAP, and DPPH and by the content of total polyphenols. With respect to their maturity stage, the ANOVA results for the antioxidant activity of both fruits evaluated show p-values <0.0001 in all cases, indicating that there are statistically significant differences between the mean of each evaluated variable and the state of maturity, with a 95% statistical confidence. These results demonstrated the state of maturity is a vital importance factor on the antioxidant activity of these fruits. The multiple range test was performed on these results using Fisher's least significant difference (LSD) with 95% confidence. The mean graphs of the LSD tests are observed in Figs. 1 and 2 for goldenberry and purple passion fruit, respectively. In Fig. 1a maturity

Table 1
Randomized lattice simplex mixture design.

Run	Components	
	Goldenberry (0–1)	Purple passion fruit (0–1)
1	0.5	0.5
2	0.25	0.75
3	0.75	0.25
4	0	1
5	0.5	0.5
6	1	0
7	1	0
8	0.5	0.5
9	0	1

Table 2
State of maturity for goldenberry and purple passion fruit Goldenberry.

Goldenberry				
State of maturity (color)				
	1	3	5	6
° Brix	8.00 ± 0.00	10.00 ± 0.00	11.33 ± 0.58	13.00 ± 0.00
Citric acid (%)	1.85 ± 0.02	1.27 ± 0.02	1.28 ± 0.11	1.28 ± 0.04
Purple passion fruit				
State of maturity (color)				
	3	4	6	
° Brix	18.33 ± 0.58	16.67 ± 0.58	13.67 ± 0.58	
Citric acid (%)	5.76 ± 0.64	3.73 ± 0.35	2.55 ± 0.12	

stage 1 is shown with a higher antioxidant activity quantified by ABTS with statistically significant differences with respect to the other 3 maturity stages, in Fig. 1b maturity stage 6 is shown with a higher antioxidant activity quantified by DPPH with statistically significant differences with respect to the other 3 maturity stages, and in Fig. 1c and d, maturity stage 3 is shown with a higher iron reducing capacity (5,69 ± 0,27 μMTrolox/g) and higher total polyphenol content (0,50 ± 0,024 μgAG/g) with statistically significant differences with respect to the other 3 maturity stages for the goldenberry, the values obtained were for fresh weight. This stage of maturity represents a fruit of lower commercial alue; however, it could be used in innovative technological processes and prevent waste during the entire post-harvest process. Fig. 2a, b, 2c, and 2d clearly show that maturity stage 3 for the purple passion fruit has higher antioxidant activity quantified by all the methodologies (ABTS (3,57 ± 0,19 μMTrolox/g); DPPH (4,18 ± 0,08 μMTrolox/g); FRAP (8,57 ± 0,18 μM Trolox/g)) and a higher total polyphenol concentration (1,03 ± 0,06 μg AG/g) with statistically significant

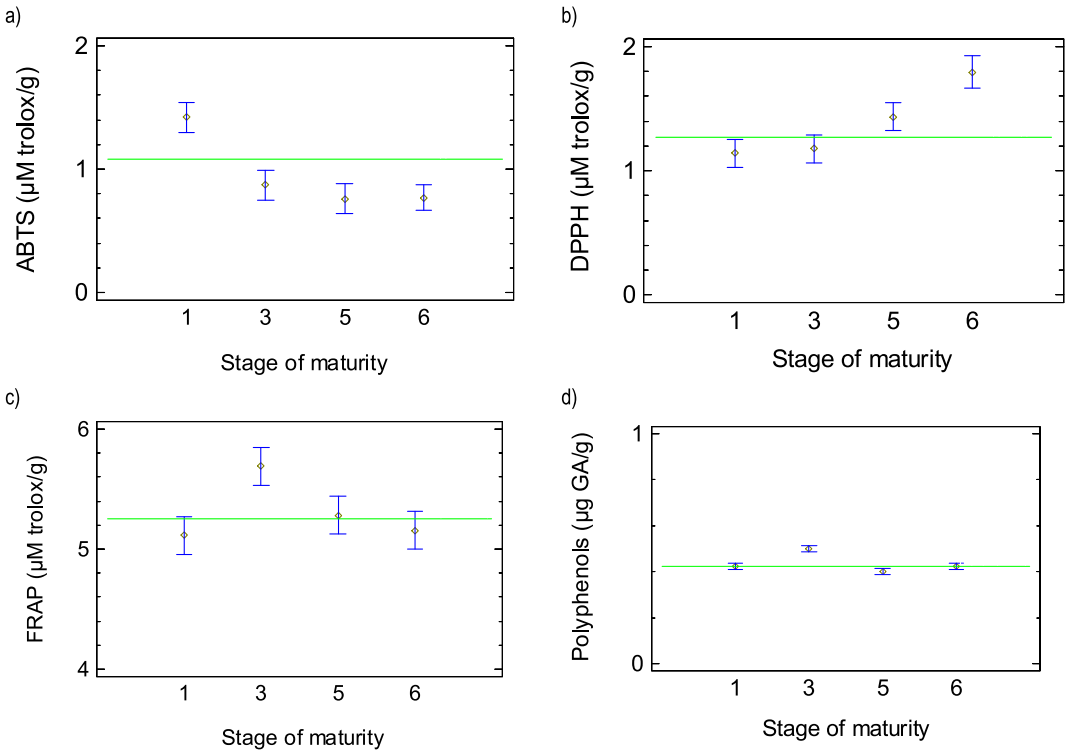


Fig. 1. Mean graphs of Fisher's LSD test for antioxidant activity quantified by ABTS (a), DPPH (b), FRAP (c), and Total polyphenols (d) with respect to the maturity stage of goldenberry.

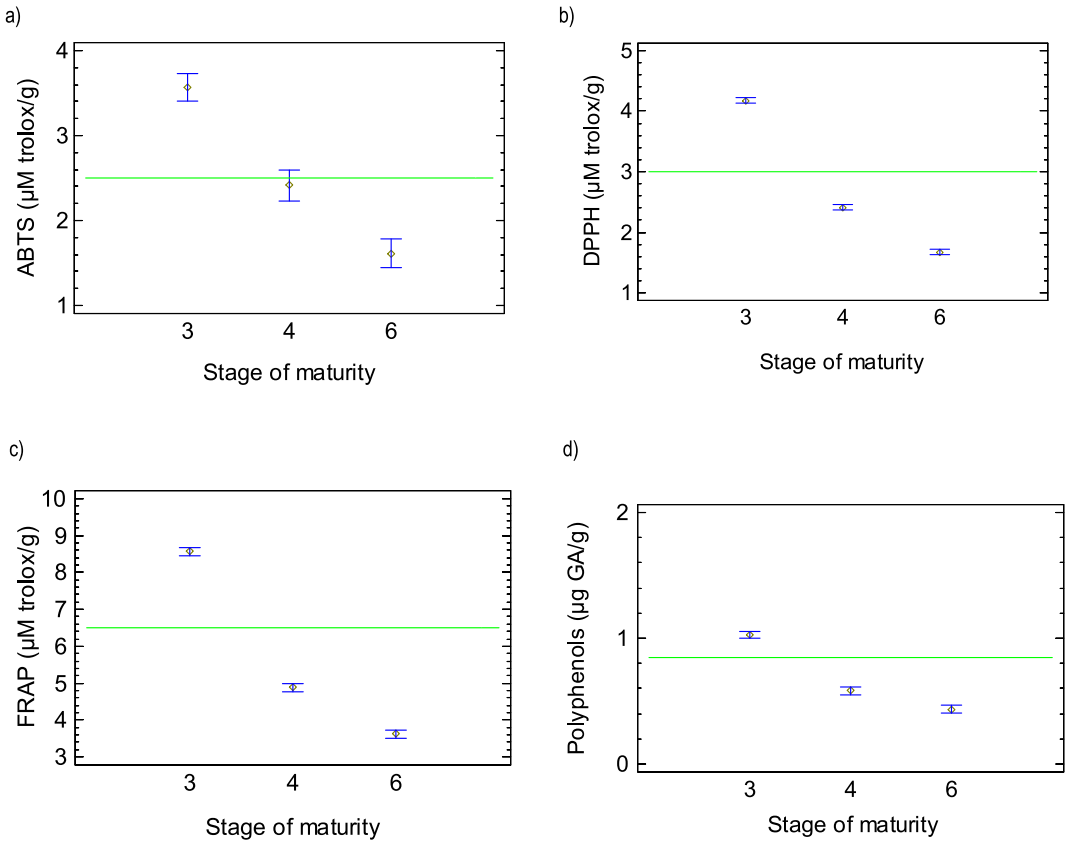


Fig. 2. Mean graphs of Fisher's LSD test for antioxidant activity quantified by a by ABTS (a), DPPH (b), FRAP (c), and Total polyphenols (d) with respect to the state of maturity of the purple passion fruit.

differences ($p < 0.0001$), these values were for fresh weight. These results were with those reported by Hou et al., 2021 [48], who evaluated the antioxidant activity (ABTS, DPPH, and FRAP), and the total content of polyphenols, and flavonoids in navel orange pulp collected at five different stages of maturity; they found that all parameters gradually decreased as the fruit was ripening. Maturity stages 3 for both goldenberry and purple passion fruit were selected to continue the research.

3.1.1. Effects of ultrasound-assisted extraction on the concentration of bioactive compounds from goldenberry

The results of the ANOVA for the goldenberry extract show p-values less than 0.05 for the total polyphenols (p-value 231 = 0.0090), with 5,29 ± 0,34 and 3,51 ± 0,55 µg AG/g, ABTS (p-value = 0.0229), with 36,53 ± 2,66 and 28,82 ± z, 59 µMTrolox/g and FRAP (p-value = 0.0087) with 34,39 ± 1,07 and 30,61 ± 0,85 µMTrolox/g for ultrasound and conventional extraction, respectively; these values were for fresh weight and results indicate statistically significant differences between the mean of these variables and the extraction process applied. Only by DPPH were significant differences with a p-value of 0.6824 not observed. The multiple range test using Fisher's least significant difference (LSD) with 95% confidence (Fig. 3a, b, 3c and 3d) indicated a higher antioxidant activity quantified by ABTS and FRAP (Fig. 3a–c). In the same way, Quintero-Quiroz et al., 2019 [49] studied the effect of ultrasound-assisted extraction of BC from annatto seeds. They found that ultrasound can speed up the extraction process, reduce energy expenses, and increase the yield process in addition to favor the antimicrobial and antioxidant activities attributed to its BC.

3.2. Physicochemical characterization

The results for the physicochemical characterization for the goldenberry freeze-dried extract for maturity stage 3 in fresh weight, were humidity 15.12 ± 0.48% w/w; fat 0.39 ± 0.51% w/w; protein 4.00 ± 0.00% w/w and ash 15.94 ± 0.00% w/w. The results coincide with those reported by Carrasco et al., 2008 [50], in Peruvian native fruits, they report fat: 0.0%; protein: 1.9%; ash.1.0%; carbohydrates 17.3% and total fiber 6.3% (w/w). For the purple passion freeze-dried extract for maturity stage 3 results were humidity 9.38 ± 0.74% w/w; fat 2.63 ± 0.42% w/w; protein 7.70 ± 0.00% w/w and ash 3.47 ± 0.00% w/w. These the results coincide with those reported by Ramos dos Reis et al., 2018 [51]. They did a physicochemical analysis for yellow orange and purple passion fruit and reported fat: 1.09 ± 0.04%. Protein: 6.53 ± 0.23%; ash: 2.95 ± 0.14%; carbohydrates: 89.42 ± 0.00% and total fiber 1.40 ± 0.18% (w/w).

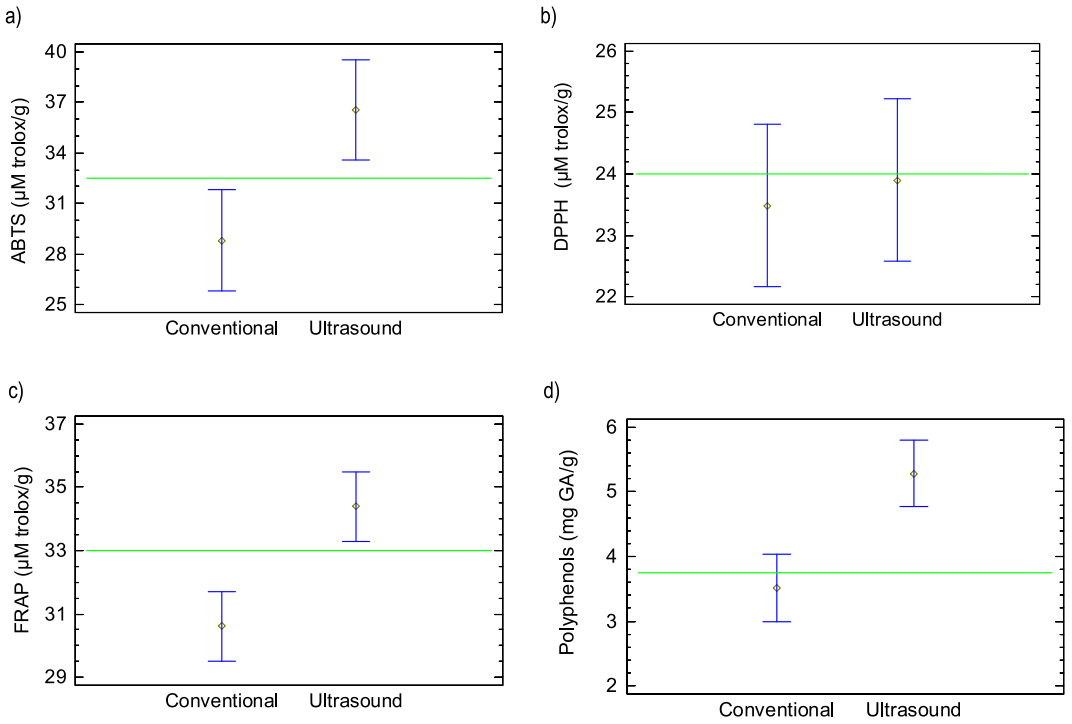


Fig. 3. Mean graphs of Fisher's LSD test for the effect of the goldenberry extraction process on antioxidant activity quantified by ABTS (a), DPPH (b), FRAP (c), and Total polyphenols (d).

3.3. *Quantification of bioactive compounds*

According to the results obtained for freeze-dried extract in fresh weight, there is a statistically significant difference between the concentration of the BC present in both fruits for both total polyphenols and total carotenoids with p-values of 0.0002 and 0.0075, respectively. Fig. 4a clearly shows that purple passion fruit has a greater concentration of carotenoid compounds and polyphenolic compounds than the goldenberries. The goldenberry has a total carotenoid content of 2057.80 ± 405.4 mg β-carotene/100 g in fresh weight, this is significantly higher than the concentration reported by Carrasco in 2008 [50], which was 0.0264 ± 0.0003 mg β-carotene/g, and by Etzbach L. et al., 2020 (118.50 µg/g dw) [52]. The observed difference in carotenoid content between the reported results and the available literature may be attributed to the potential lack of precision in spectrophotometric methods, which have a tendency to overestimate carotenoid content. The goldenberry has a total polyphenol content of 5.2862 ± 0.3359 mg GA/g (Fig. 4b), also higher than the results reported by Carrasco, 2008 [50], which was 1.54 ± 0.03 mg GA/g in freeze-dried pulp. The content of carotenoids is 3208.9 ± 367.1 µg β-carotene/mg for the purple passion fruit, a much higher result than that reported in the work carried out by Ramos dos Reis et al., 2018 [51], which was 2.8856 ± 0.0003 µg/g Purple passion fruit has a total polyphenol content of 9.1168 ± 0.3676 mg GA/g. This result is greater than that reported by Carmona-Hernandez et al., 2019 [24], of 1.62 ± 0.09 mg GA/g for an extract obtained with methanol at 70%.

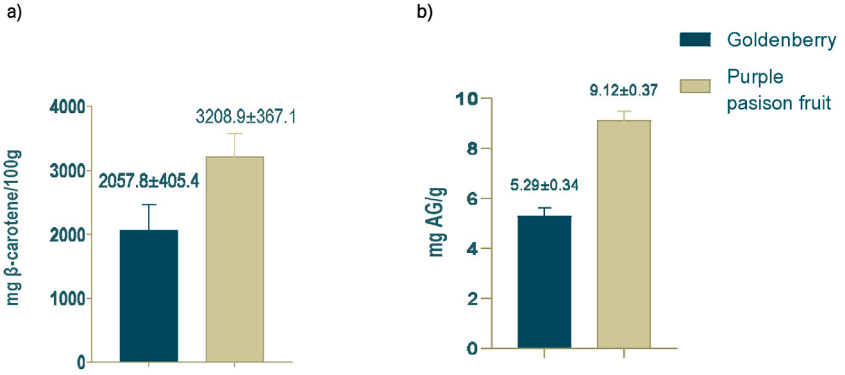


Fig. 4. Total carotenoids (a) and Total polyphenols concentration (b) for both fruits.

3.4. Identification by HPLC-MS of carotenoids present in fruits

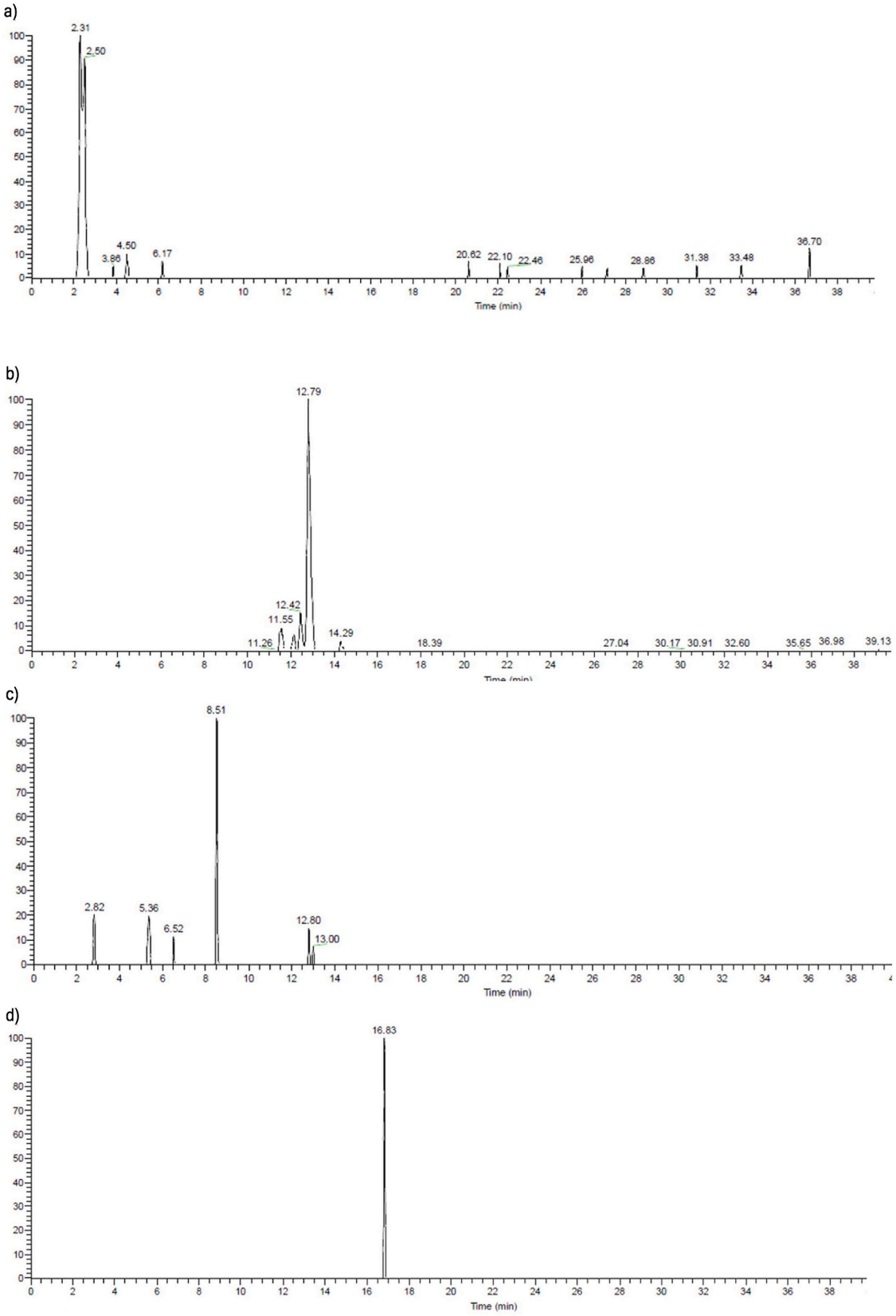
Table 3 shows the 14 carotenoids identified by HLPC-MS for both studied fruits: lutein 5.6-epoxide taraxanthin (Fig. 5a), lutein (13Z)-lutein and/or (13′Z)-lutein zeinoxanthin, α-carotene, β-carotene (Fig. 5b), cryptoxanthin (Fig. 5c), phytoene, phytofluene, lycopene, α-cryptoxanthin myristate all (E) isomers, antheraxanthin all (E) isomers, violaxanthin all (E) isomers and violaxanthin palmitate. Only the violaxanthin dipalmitate all (E) isomers (Fig. 5d) are present in purple passion fruit without being present in the goldenberry. These results are similar to the study carried out by Etzbach et al., 2018 [15], who previously reported 53 carotenoids present and 42 tentatively identified in goldenberry fruits at different ripening states and in different fruit fractions by HPLC-DAD-APCI-MSn. According to Etzbach et al., 2018 [15], the carotenoid composition of the goldenberry was dominated by β-carotene all (E) isomers. Despite the lack of reports on the carotenoids in purple passion fruit, there are some described for passion fruit. Pertuzatti et al., 2015 [53] reported the presence of β-carotene, lycopene β-criptoxanthin, and lutein in yellow passion fruit. It was also reported by Ramos dos Reis et al., 2018 [51].

3.5. Antioxidant activity of freeze-dried extracts

The ANOVA results comparing the antioxidant activity of the freeze-dried extracts in fresh weight for maturity stage 3 in both fruits showed p-values less than 0.05 for antioxidant activity quantified by ABTS (p-value = 0.0442), DPPH (p-value = 0.0007), TBARS (p-value = 0.0000), and ORAC (p-value = 0.0057). These values indicate statistically significant differences between the mean of these variables evaluated in each extract with a 95% statistical confidence level. However, for the capacity of the samples to reduce the ferric iron FRAP, the p-value was 0.8390. That indicates that there are no statistically significant differences between the evaluated extracts. Furthermore, Fig. 6a shows the goldenberry has antioxidant activity greater than that of purple passion fruit, quantified by ABTS and DPPH. These results indicate the goldenberry has a greater capacity to trap free radicals than purple passion fruit. The DPPH value for the goldenberry was 36.5282 ± 2.6632 μM Trolox/g, which is much higher than the value reported by Oztruk et al., 2017 [54] (5.5208–6.0469 μM Trolox/g) for deferent type of goldenberries classified according to the cultivation area. The value for purple passion fruit was 23.9005 ± 0.9574 μM Trolox/g, higher compared to that reported by Puente et al., 2020 [55], which was 2.1082 ± 0.0945 μM Trolox/g for freeze-dried pulp. The ORAC for goldenberry was 23.02 ± 0.36 μM Trolox/g and for purple passion fruit 32.44 ± 0.94 μM Trolox/g. In this case, purple passion fruit has greater antioxidant capacity than goldenberry, these results may be attributed to the fact that the ORAC methodology is the only method evaluated that is based on the transfer of the hydrogen atom (HAT) to measure ability of an antioxidant to eliminate free radicals by donating a hydrogen atom. Furthermore, TBARS quantifies malondialdehyde (MDA) as the main product of hydroperoxides degradation generated by lipid oxidation. Fig. 6b shows that the goldenberry generates less MDA than purple passion fruit. This fact implies that goldenberries also have a greater capacity of inhibiting lipid oxidation. Although the goldenberry has a greater antioxidant activity than the purple passion fruit, the purple passion fruit has a higher concentration of polyphenolic compounds and carotenoids, this may be due to the presence of other compounds that exhibit antioxidant activity, such as vitamin C, since the goldenberry has a higher content of vitamin C, some authors such Bazalar Pereda et al., 2019 report 33.35 ± 0.37 mg Ascorbic acid/100 g fresh weight in goldenberries cultivated in Argentinean Northern Andean region [56] while Ramadan, 2011 reports 46 mg/100 g in fresh weight [57], on the other hand for purple passion fruit Franco et al., 2014 report 24.4 mg of ascorbic acid/100 g of fruit for Colombian goldenberry 7 days after being harvested [58].

Table 3
Carotenoids present in extracts of goldenberry and purple passion fruit.

Compound	Molecular formula	Molecular weigh	Molecular weight (MS/MS)	Purple passion fruit	Retention time (min)	Goldenberry	Retention time (min)
Lutein	C40H56O2	568.9	568.42748	X	2.61	X	2.67
Lutein 5.6-epoxide Taraxanthin	C40H56O3	584.9	584.4224	X	2.56	X	2.31
Zeinoxanthin	C40H56O	552.9	552.43257	X	8.51	X	8.49
Cryptoxanthin	C40H56O	552.9	552.43257	X	8.51	X	8.49
α-Carotene	C40H56	536.9	536.43765	X	12.83	X	12.79
Phytoene	C40H64	544.9	544.50025	X	3.21	X	3.12
Phytofluene	C40H62	542.9	542.4846	X	4.36	X	25.25
β-carotene	C40H56	536.9	536.43765	X	12.82	X	12.79
Lycopene	C40H56	536.9	536.43765	X	12.82	X	12.79
(all-E)-α-Cryptoxanthin myristate	C54H82O2	763.2	762.63093	X	3.18	X	1.96
(all-E)- Antheraxanthin	C40H56O3	584.9	584.4224	X	2.56	X	2.53
(13Z)-Lutein and/or (13′Z)-lutein	C40H56O3	584.9	584.4224	X	2.56	X	2.53
(all-E)- Violaxanthin	C40H56O3	584.9	600.41731	X	5.06	X	2.24
Violaxanthin palmitate	C70H112O6	1049.6	1048.84534	X	4.13	X	37.46
(all-E)-Violaxanthin Di-palmitate	C72H116O6	1077.7	1076.87664	X	16.83		



(caption on next page)

Fig. 5. Identified carotenoid chromatograms for a) Lutein 5.6-epoxide taraxanthin and, b) β-Carotene in goldenberry; and c) Cryptoxanthin and d) (all-E)-violaxanthin Di-palmitate in purple passion fruit.

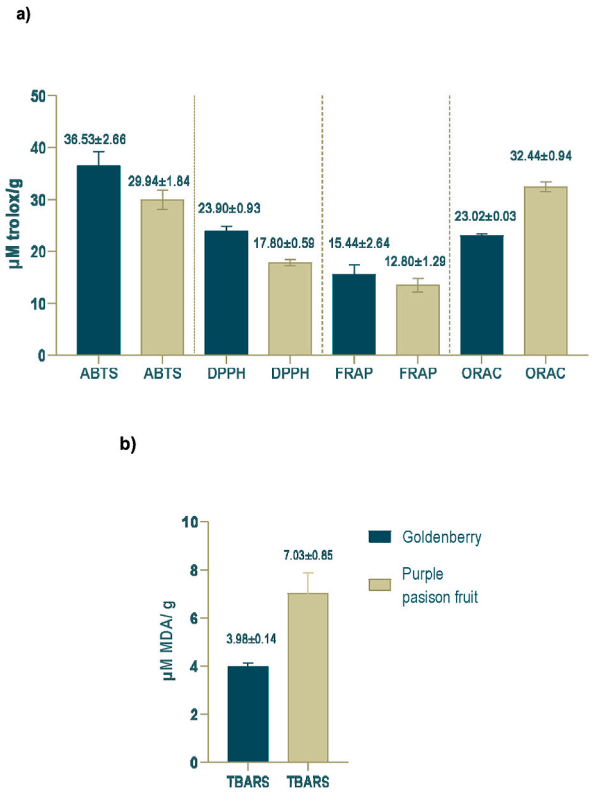


Fig. 6. Antioxidant activity of freeze-dried extracts quantified by a) ABTS. DPPH, FRAP and ORAC and b) TBARS.

3.6. Fruit mixture design

To define the optimal mixture of the fruits to be used, an experimental design of the two-component randomized lattice simplex mixture type was carried up: goldenberry (0–1) and purple passion fruit (0–1) (Table 4). The experimental design was performed in STATGRAPHICS 19. The p-values for each of the response variables (less than 0.05) except for TBARS indicate that these components of the mixture have a statistically significant effect on all of them with 95% statistical confidence. The mathematical models that describe the effect of the mixture on each of the response variables are described in equations (1)–(4) for ABTS, DPPH, FRAP, and ORAC, respectively, and an r2-adjusted above 75% in all cases except for TBARS. Fig. 7c (FRAP) has a linear behavior that agrees with equation (3), where the quadratic and cubic variables are practically insignificant compared to the other two linear variables.

Table 4
Experimental mix design.

RUN	Components		Response variables				
	(A) Goldenberry (0–1)	(B) Purple passion fruit (0–1)	ABTS (µM trolox/g)	DPPH (µM trolox/g)	FRAP (µM trolox/g)	TBARS (µM MDA/ 100 g)	ORAC (µM trolox/ 100 g)
1	0.5	0.5	13.56	14.27	18.82	137.09	2914.23
2	0.25	0.75	13.01	11.73	17.61	134.57	3118.46
3	0.75	0.25	13.03	16.05	19.95	63.11	2632.20
4	0	1	10.99	8.08	16.99	94.02	3310.50
5	0.5	0.5	13.21	14.37	17.47	105.95	2962.56
6	1	0	13.29	16.58	21.66	122.28	2328.22
7	1	0	15.21	18.55	19.52	68.45	2276.61
8	0.5	0.5	13.74	12.69	19.11	124.61	2453.88
9	0	1	10.19	7.86	16.06	114.30	3177.06
p-value			0.0126	0.0001	0.0009	0.2230	0.0176
r2			86.64%	96.24%	81.28%	55.39%	84.68%
r2-adjusted			78.62%	94.98%	78.60%	28.62%	75.50%

$$ABTS = 14.2301 * A + 10.5705 * B + 4.11275 * A * B - 9.66613 + *A * B * (A - B) \quad (1)$$

$$DPPH = 17.5944 * A + 8.00635 * B + 4.42383 * A * B - 2.55012 * A * B * (A - B) \quad (2)$$

$$FRAP = 20.6136 * A + 16.5499 * B - 0.0458133 * A * B + 1.6268 * A * B * (A - B) \quad (3)$$

$$ORAC = 2311.25 * A + 3252.61 * B + 121.184 * A * B - 83.08 * A * B * (A - B) \quad (4)$$

3.6.1. Mixture optimization

The optimization of multiple responses was done to maximize the antioxidant activity quantified by ABTS, DPPH, FRAP, and ORAC and to minimize the concentration of MDA considering that it is inversely proportional to the antioxidant activity quantified by TBARS. The combination of factor levels that maximizes the 'desirability' function is reached with goldenberry 0.83 and purple passion 0.17 (desirability = 0.97). However, Fig. 7 shows the trace plots are for a reference blend (0.5–0.5) at the intersection of the lines. The goldenberry component is represented by a blue line and the purple passion fruit component by a red line in all cases. Fig. 7a, b, and 7c show as the proportion of goldenberry (blue line) in the mixture increases, the antioxidant activity, quantified by ABTS, DPPH, and FRAP, respectively, also increases. In the case of ORAC, Fig. 7d, the red line (purple passion fruit) indicates that as its proportion increases in the mixture, the antioxidant activity increases.

These results are consistent with those presented above, where the goldenberry has an antioxidant activity greater than the purple passion fruit quantified by ABTS, DPPH, and TBARS, and the purple passion has an antioxidant activity greater than goldenberry quantified by ORAC. According to Wang et al., 2011 [27], a mixture of different foods or plant extracts can exhibit additive synergistic or antagonistic interactions between their different BC. Antagonism occurs when the sum of the effects is less than the predicted mathematical sum. For example, these antagonistic effects may have negative health implications for people trying to increase antioxidant intake by consuming mixed foods. This behavior is observed in runs 1 and 5 for the DPPH response variable, where the sum of the expected antioxidant activities should be 12.77 ± 0.62 (μM trolox/g), but they exhibit a result of 14.27 and 14.37 (μM trolox/g), respectively. The other response variables show similar results, except for FRAP which should be 18.56 ± 1.08 (μM trolox/g) and runs 1 and 5 of the experimental mixture design show values of 18.82 and 17.47 (μM trolox/g), respectively, which implies an additive behavior. Wang et al., 2011 [27], evaluated the antioxidant activity in vitro by DPPH, FRAP and ORAC, and total polyphenol content of methanolic extracts from 11 foods: three fruits (raspberries, blackberries and apples), four vegetables (broccoli, tomatoes, mushrooms, and purple cauliflower) and four legumes (soybeans, adzuki beans, kidney beans and black beans). These extracts were evaluated individually and then in binary mixes in 1:1 ratio for a total of 55 mixes. The results demonstrated that different food categories exhibit a different antioxidant potential, the group of vegetables had the lowest antioxidant capacity compared to fruits or legumes groups, the three types of interactions (synergistic, additive, and antagonistic) were observed; the combination of foods within the same food

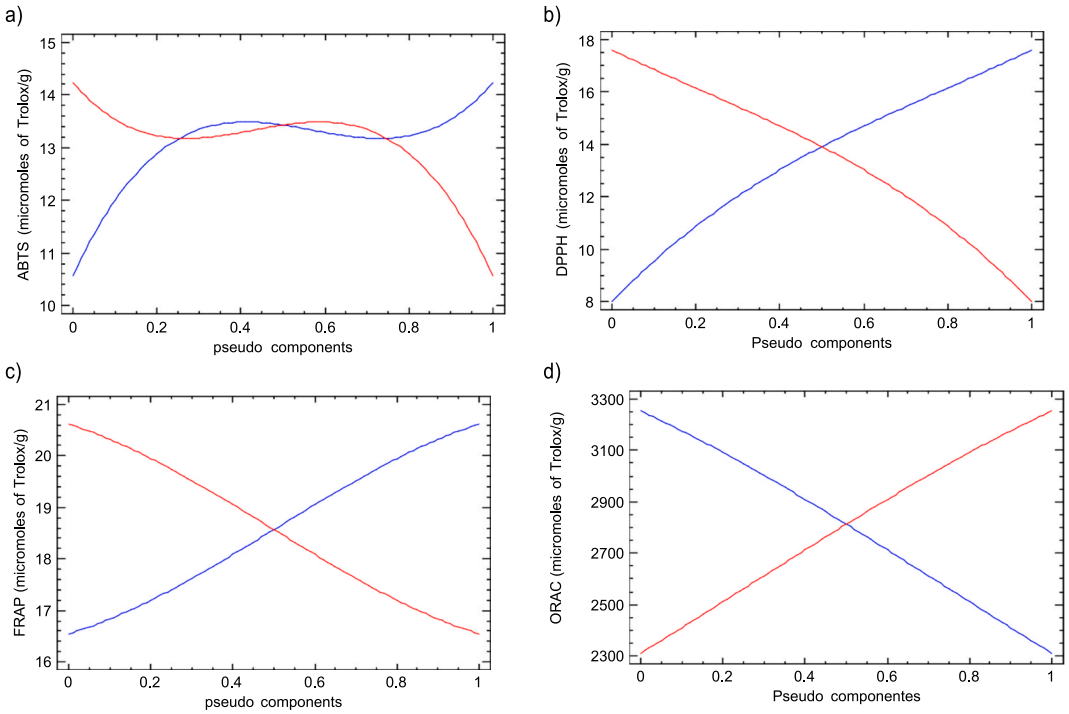


Fig. 7. Trace plot reference blend (0.5–0.5) for ABTS (a), DPPH (b), FRAP (c), and ORAC (d).

category showed that the 13% have synergistic interactions, the 68% additive interactions and 21% antagonistic interactions, while the combination of foods between the food categories 21% showed synergistic interactions, 54% interactions additive, and 25% antagonistic interactions. Thus, combinations from across food categories are more likely (21%) to demonstrate synergistic interactions compared to combinations within a category (13%). These results demonstrate that interactions between antioxidants can not only produce positive effects but also negative effects as they concluded that the combination of antioxidant foods does not guarantee that the antioxidant activity will be equal to the expected value. In another study conducted by Schiassi et al., 2020 [28], who wanted to optimize a mix of berries juice made with coconut water, using mixture design and response surface methodology for desirability function, as well as to verify the influence of the fruits on the physical, physicochemical, nutritional factors and sensory characteristics of the final product; the components of the mixtures were strawberry (Fragaria x ananassa), blackberry (Rubus spp.), red raspberry (Rubus idaeus) sucralose, and green coconut water (Cocus nucifera L.) with established proportions of 50% mix and 50% coconut water and 0.05 g of sucralose for every 1 L of juice. The results showed that the formulation composed of blackberry and red raspberry (0.5–0.5) presented the highest antioxidant activity quantified by ABTS and DPPH and a higher content of phenolic compounds and anthocyanins, even higher than individual fruit juices, demonstrating a possible synergistic effect.

4. Conclusion

The results demonstrated the state of maturity is a vital importance factor on the antioxidant activity of these fruits, maturity stage 3 was selected in both fruits for the analysis. For the case of the goldenberry, this stage of maturity presented a significantly higher content of total polyphenols (0,50 ± 0,024 μgAG/g) and iron reducing capacity (5,69 ± 0,27 μMTrolox/g) (FRAP) compared to other stages of maturity; and for the case of the purple passion fruit, this stage of maturity had the highest polyphenols content (1,03 ± 0,06 μg AG/g) and the highest antioxidant activity by all methodologies evaluated (ABTS (3,57 ± 0,19 μMTrolox/g); DPPH (4,18 ± 0,08 μMTrolox/g); FRAP (8,57 ± 0,18 μMTrolox/g)). The goldenberry had a higher antioxidant activity than the purple passion fruit, quantified by ABTS (36.53 ± 2.66), DPPH (23.90 ± 0.93), and TBARS (3.98 ± 0.14 μM MDA/g). However, the purple passion fruit had a higher content of bioactive compounds (32.09 ± 3.67 μg β-carotene/mg and (9.12 ± 0.37 mg AG/g) and a higher antioxidant activity quantified by ORAC (32.44 ± 0.94 μMTrolox/g). Although the goldenberry has a greater antioxidant activity than the purple passion fruit, the purple passion fruit has a higher concentration of polyphenolic compounds and carotenoids, this may be due to the presence of other compounds that exhibit antioxidant activity, such as vitamin C. The presence of some carotenoids in the fruits was identified. Some of the most relevant were β-carotene, cryptoxanthin, phytoene, phytofluene, and the presence of violaxanthin dipalmitate in purple passion fruit. The mixture of these fruits had a synergistic effect, with the highest antioxidant activity observed with the mixture composed of 83% goldenberry and 17% purple passion fruit.

Author contributions

A.M.N.-D. performed the experimental part and wrote the manuscript. J.Q.-Q. and G.L.C.-G advised the design of experiments and reviewed and corrected the manuscript. G.L.C.-G is the project administrator, and finally, MJBA and JCCC reviewed and corrected the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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List of abbreviations

HPLC-MS	High Performance Liquid Chromatography-Mass Spectrometry
NCD	Chronic Non-Communicable Diseases
CVD	Cardio-Vascular Disease
LDL	Low-Density Lipoprotein
BHT	Butylated-hydroxytoluene
IYFV	International Year of Fruits and Vegetables
USD-FOB	Dollars Free On Board
BC	Bioactive compounds
NTC	Colombian Technical Standards
MDA	Malondialdehyde

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