

Celebrating the International Year of Fruits and Vegetables Nutritional Quality and Nutraceutical Potential of Fruits and Vegetables as a tool for Genetic Breeding Programs

Editor

Maximiliano Dini Instituto Nacional de Investigación Agropecuaria (INIA), Programa Nacional de Investigación en Producción Frutícola, Estación Experimental INIA Las Brujas, Canelones, Uruguay.

> Guillermo A. Galván[®] Universidad de la República, Facultad de Agronomía, Departamento de Producción Vegetal, Canelones, Uruguay.

Correspondence

F. Ibañez fibanez@inia.org.uy

Received 25 May 2021 Accepted 20 Sep 2021 Published 05 Jan 2022

Citation

Ferrari V, Rodríguez G, González M, Vicente E, Giménez G, Cabrera D, Ibañez F. Nutritional Quality and Nutraceutical Potential of Fruits and Vegetables as a tool for Genetic Breeding Programs. Agrociencia Uruguay [Internet]. 2021 [cited dd mmm yyyy];25(NE2):e814. Available from: http://agrocienciauruguay. uy/ojs/index.php/agrociencia/art icle/view/814. Calidad nutricional y potencial nutracéutico de frutas y hortalizas como herramientas para el mejoramiento genético

Qualidade Nutricional e Potencial Nutracêutico de Frutas e Vegetais como Ferramentas de Melhoramento Genético

Ferrari, V.¹; Rodríguez, G.²; González, M.²; Vicente, E.²; Giménez, G.²; Cabrera, D.³; Ibañez, F.¹

¹Instituto Nacional de Investigación Agropecuaria (INIA), Plataforma Agroalimentos, Canelones, Uruguay

²Instituto Nacional de Investigación Agropecuaria (INIA), Programa de Investigación Producción Hortícola, Salto, Uruguay

³Instituto Nacional de Investigación Agropecuaria (INIA), Programa de Investigación Producción Frutícola, Canelones, Uruguay



Abstract

Prevention of the development of chronic diseases related to metabolic syndrome has been found to depend on a healthy diet. Among healthy foods, fruits and vegetables play a very important role due to their nutrient content, especially antioxidants, vitamins and polyphenols. For 20 years, INIA has promoted the concept of guality associated to the differentiation of products in the fruit and horticultural sectors, considering aspects not only linked to the organoleptic qualities, but also the production processes that ensure food safety and nutritional contribution. More recently, research works have been implemented to contribute to highlighting the role of fruits and vegetables as nutraceutical foods, contributing to the prevention of chronic non-transmissible human diseases such as diabetes, cancer, or obesity. Also, to contribute to the knowledge of the effects of factors as breeding and culture management on the nutritional potential and promote production, consumption and commercialization. The bioactive compounds content and in vitro antioxidant activity (DPPH and ORAC) were characterized in different genotypes of native fruits, onion, potato, strawberry, sweet potato and tomato. High levels of flavonoids (quercetin) were found in onions and advanced lines. Samples of tomatoes, potatoes, sweet potatoes, and strawberries showed high contents of phenolic compounds, ascorbic acid, anthocyanins, total carotenoids, and total antioxidant capacity (ORAC) with a great interspecies variability; so, they can be included in the development of varieties differentiated by nutraceutical attributes. The analyses conducted also demonstrate the potential of native fruits as sources of essential nutrients, with contents comparable to and even superior to other fruits considered "superfruits". Through selection and breeding, national varieties can be developed with outstanding organoleptic characteristics, good levels of bioactive compounds, and antioxidant properties that contribute to the health of the population.

Keywords: nutrition, antioxidants, ORAC, genetic breeding

Resumen

Se ha encontrado que la prevención del desarrollo de enfermedades crónicas relacionadas con el síndrome metabólico depende de una dieta saludable. Entre los alimentos saludables, las frutas y las hortalizas juegan un papel muy importante por el contenido de nutrientes, sobre todos del tipo antioxidantes, vitaminas y polifenoles. Desde hace 20 años INIA ha impulsado en los rubros hortifrutícolas el concepto de calidad asociado a la diferenciación de los productos considerando aspectos no solo vinculados a las cualidades organolépticas, sino también a los procesos de producción que aseguren la inocuidad y el aporte nutricional. Más recientemente se han realizado trabajos de investigación que contribuyen a resaltar el rol de las frutas y las hortalizas como alimentos nutraceúticos; preventivos de enfermedades humanas crónicas no transmisibles como diabetes, cáncer u obesidad. También contribuir al conocimiento de los efectos de factores como el mejoramiento genético y el manejo cultural, y promover la producción, el consumo y la comercialización. El contenido de compuestos bioactivos y la actividad antioxidante in vitro (DPPH y ORAC) se caracterizaron en diferentes genotipos de frutos nativos, cebolla, papa, frutilla, boniato y tomate. Se encontraron altos niveles de flavonoides (quercetina) en cebollas y sus líneas avanzadas. Muestras de tomates, papas, boniatos y frutillas presentaron altos contenidos de compuestos fenólicos, ácido ascórbico, antocianinas, carotenoides totales y la capacidad antioxidante total (ORAC) con una gran variabilidad interespecie; por lo que pueden incluirse en el desarrollo de variedades diferenciadas por atributos nutracéuticos. Los análisis realizados demuestran también el potencial de las frutas nativas como fuentes de nutrientes esenciales, con contenidos comparables e incluso superiores a otras frutas consideradas «superfrutas». Mediante selección y mejoramiento genético se pueden desarrollar variedades nacionales con características organolépticas destacadas, buenos niveles de compuestos bioactivos y propiedades antioxidantes que contribuyen a la salud de la población.

Palabras clave: nutrición, antioxidantes, ORAC, selección genética



Resumo

A prevenção do desenvolvimento de doenças crônicas relacionadas à síndrome metabólica depende de uma dieta saudável. Dentre os alimentos saudáveis, frutas e hortaliças desempenham um papel fundamental devido ao conteúdo de nutrientes, principalmente antioxidantes, vitaminas e polifenóis.

Há 20 anos, o Instituto Nacional de Investigación Agropecuaria (INIA) promove a gualidade associada à diferenciação de produtos no setor de frutas e legumes. O conceito de gualidade foi ampliado a aspectos que levam em consideração não só as qualidades organolépticas e de sabor, mas também os processos de produção que garantem a segurança alimentar e o aporte nutricional. Mais recentemente, se agregou a esses temas a implementação de trabalhos de pesquisa que contribuem para evidenciar o papel das frutas e hortaliças como alimentos preventivos de doenças humanas (Alimento Funcionais, Nutracêuticos). Com o objetivo de contribuir para o conhecimento e promoção do consumo, iniciou-se o trabalho de pesquisa de potencial nutricional das principais variedades e seleções avançadas geradas pelos programas de melhoramento hortícola e frutícola do INIA. O teor de micronutrientes e a atividade antioxidante in vitro (DPPH e ORAC) foram caracterizados em variedades e seleções de morango, frutas nativas, cebola, batata, tomate e batata doce. Altos níveis de flavonoides (guercetina) foram encontrados em cebolas INIA e linhas avançadas. No tomate, batata, batata doce e morango, destaca-se que os compostos fenólicos, vitamina C, antocianinas, carotenoides totais e capacidade antioxidante total (ORAC) apresentam grande variabilidade, podendo ser incluídos no desenvolvimento de variedades diferenciadas por atributos nutracêuticos. As análises realizadas também demonstram o potencial das frutas nativas como fontes de nutrientes essenciais, com teores comparáveis e até superiores a outras frutas consideradas "superfrutas". Através da seleção e do melhoramento genético, variedades nacionais com excelentes características organolépticas, bons níveis de micronutrientes e com propriedades antioxidantes que contribuem para a saúde podem ser desenvolvidas.

Palavras-chave: nutrição, antioxidantes, ORAC, seleção genética.

1. Introduction

The health-promoting properties of fruits and are well stablished vegetables in many epidemiological investigations. Lower risk of chronic diseases due to fruits and vegetables consumption is associated mainly to the dietary fiber and bioactive compounds contents. Specifically, some of these compounds have high antioxidant activity that avoid oxidative stress, not only in vegetable tissues, but also in animals through the digestion. It is therefore important to recognize which fruits and vegetables have the highest contents with more antioxidant capacity potential, for improving their quality and introducing them regularly into the diet. Basal information on bioactive compounds in fruits and vegetables in several countries or governmental regulatory services is published on food data composition tables⁽¹⁾⁽²⁾. Phenolic compounds comprise a major group⁽³⁾. Due to a wide variety of characterization is difficult in complex matrices. Nevertheless, flavonoids have been studied extensively recently and research has repeatedly found that foods with high content of them correlate with better health indicators⁽⁴⁾⁽⁵⁾. Flavonoids, like anthocvanins and guercetin, and carotenoids have different biochemical properties, mainly because the first are hydrophilic and the latter lipophilic. These compounds may contribute not only to improve color or visual aspect, but also to organoleptic characteristics, effect of cooking methods or industrialization procedures. In regard to health benefits, the contribution is also diverse, and many reviews address the investigations in the area. Effects of groups of compounds are described in general: anthocyanins⁽⁶⁾, carotenoids⁽⁷⁾ and flavonoids⁽⁸⁾; as well as for micronutrients and/or action mechanism specifically. Even in pandemic situation, the role of

molecules that are included in this group, individual



quercetin, for example, in prevention and treatment of SARS-CoV-2 related disease was recently reported.⁽⁹⁾⁽¹⁰⁾

Antioxidant capacity, as a predictor of the nutraceutical potential of food, can be quantified through the antioxidant activity in vitro analysis. The most largely used are the following: antioxidant capacity with 2,2'-azino-bis-(3ethylbenzothiazoline-6-sulfonic acid) (TEAC/ABTS); radical scavenging capacity ferric ion reducing antioxidant power (FRAP), 2,2diphenyl-picrylhydrazyl (DPPH), and oxygen radical absorbance capacity (ORAC). The ORAC analysis demonstrated some technical advantages and may be more accurate and specific⁽¹¹⁾. However, for antioxidant characterization overall it is recommended to employ more than one in vitro assay when complex biochemical matrices are investigated. Finally, assessing in this way the antioxidant capacity of a given food does not imply that the total activity of its antioxidant compounds will become bioavailable to the organism and to

benefit health. So, determining the antioxidant bioactive compounds content and antioxidant capacity in fruit and vegetables is critical for understanding their potential dietary benefit and/or the effect of several factors, such as genetics, environmental, cultural management and industrial processing, on them. To the best of our knowledge, this study represents a comprehensive screening of micronutrient composition and antioxidant capacity of fruit and vegetables under investigation in Uruguay.

2. Materials and methods

2.1 Fruit and vegetable samples

Different genetic varieties or selections of fruit and vegetables were sampled according to Table 1. All of them belong to the Breeding Programs at the National Institute for Agricultural Research (INIA) in two locations: Las Brujas and Salto Grande. Native fruit orchards were conducted only at Las Brujas (W. Ferreira Aldunate Station) in southern Uruguay.

Name		Botanical nam	Total genotypes (selections)	
Arazá, Strawberry guava	red fruits	Psidium cattleianum Sab.	f. cattleyanum	3 (3)
	yellow fruits		f. lucidum	3 (3)
Guaviyú		Myrcianthes pungens (Berg.) Legr.		3 (3)
Guayabo del País, Feijoa		Acca sellowiana (Berg.) Burret		3 (2)
Onion		Allium cepa L.		8 (1)
Potato		Solanum tuberosum L.		7 (4)
Strawberry		Fragaria x ananassa Duch.		10 (7)
Sweet potato		Ipomoea batatas L. Lam.		6 (1)
Tomato		Solanum lycopersicum L.		19 (13)

Table A	0 - 1 + +		£	
Table 1.	Selected truits	and vegetables	Trom IINIA	Breeding Programs

The samples were harvested from plants that had great performance in terms of production and sanity, at commercial maturation range. A representative amount of approximately 2-3 Kg of

fruit and vegetable of each genotype in three repetitions was collected and transported to laboratory. The arazá and guaviyú native fruits were separated in edible parts and seeds, and the edible parts were homogenized using a blender during 1 min. The homogenate was immediately frozen at -80 °C until micronutrient and antioxidant analyses. As regards guayabo del país, onion and sweetpotato, samples were peeled before homogenization.

2.2 Bioactive compounds analyses

The homogenates samples (0.2 g) were mixed with 1 mL of methanol/water solution (80:20, v/v) to micronutrient extraction. The samples were placed in an ultrasonic bath during 10 min at 20 °C and stored at 20 °C avoiding light exposure. Then they were centrifugated at 12,500 rpm during 5 min, and the methanolic extract was diluted if necessary and used for subsequent analyses. L-Ascorbic acid (corresponding to vitamin C) and total phenolic content were evaluated according to integrated microplate method⁽¹²⁾. In brief, 15 μ L of methanolic extract were diluted with 240 µL milli-Q ultrapure water, and 15 µL of Folin-Ciocalteu reagent was added. The 96 well microplate was agitated in a multi-mode microplate reader with two independent dispensers Synergy HT1 (BioTek Instruments Inc., Winooski, VT) and incubated for 3 min. The absorbance at 765 nm was determined and the vitamin C concentration estimated against ascorbic acid standard curve. The results were expressed as mg of ascorbic acid per 100 g of fresh fruit or vegetable weight (FW). The assay was continued by adding Na₂CO₃ in the microplates wells and incubated for 2 h at room temperature in the dark to determine total phenolic content in the samples. The absorbance at 765 nm was measured again and values were compared to gallic acid standard calibration curve. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g FW.

Anthocyanins content estimation was based on a pH differential method described previously⁽¹³⁾ and modified according to Ferrari and others⁽¹⁴⁾. In 96 well microplates 40 μ L of methanolic extract was diluted with 200 μ L of potassium chloride pH 1.0 (0.025 M) or sodium acetate pH 4.5 (0.4 M) buffers. Absorbance values at 520 nm and 700 nm were used to calculate anthocyanins concentration as mg cyanidin 3-glucoside per 100 g FW.



For quercetin determination 1.0 g of homogenized sample were mixed with 4.0 mL of acidified methanol:water solution (80:20; HCI 1.98 N). Tubes were vortexed and incubated in bath at 90 °C for 30 min. After decantation, the supernatant was transferred to other tube and final volume adjusted to 5.0 mL. The extracts were neutralized (NaOH 1.0 N) and 100 μ L aliquots dispensed in 96 wells microplates. Milli-Q water (150 µL) and 50 µL AICI3 (10 %) were added to color development. Absorbance at 420 nm was measured using the same microplate reader described before. The quercetin content was calculated based on a calibration curve established with the same analytical standard. The results were expressed as mg quercetin per 100 g FW.

Carotenoids content was estimated as previously reported⁽¹⁵⁾, with minor modifications. Briefly, 1.0 g of homogenized samples were mixed with 5 mL acetone/BHT (0.1 %) and vortex. Then the solutions were sonicated for 10 min and centrifugated at 10.000 rpm. The supernatant was transferred to other tube, the extraction step was repeated twice, and the final volume adjusted to 25 mL. Absorbance was measured at 450 nm (A450).

The carotenoids concentrations (C) were estimated from equation 1.

C = A450 * 537 * 1000/140663 (Eq. 1)

Calculation was based on Lambert law with β carotene molar weight (537 g.mol⁻¹) and extinction coefficient (140.663 M⁻¹.cm⁻¹). The results were expressed as mg β -carotene per 100 g FW.

2.3 Antioxidant *in vitro* analyses

The same extraction procedure in methanol:water solution (80:20 v/v) was used to obtain methanolic extracts for the DPPH and ORAC assays.

The quantification of free radical-scavenging of DPPH (2,2-diphenyl-1-picrylhydrazil) was performed mixing an aliquot of appropriated diluted extract with DPPH solution (125 mM) in methanol:water (80:20, v/v) in 96-well microplates. The mixture was shaken and incubated for 24 h at 20 °C in the dark. The reduction of absorption was measured at



517 nm and quantified using a Trolox standard curve. The results were expressed as μ mol Trolox equivalents (TE).100 g ⁻¹ FW.

The hydrophilic ORAC analysis was conducted according to methodology described by Held⁽¹⁶⁾ and Ou and others⁽¹⁷⁾ with some modifications. Briefly, an aliquot of the diluted methanolic extracts or standards solutions of Trolox (0 to 100 µM) were transferred to 96 well-microplates. The plate was placed in the microplate reader and fluorescein solution (0.008 µM) prepared with 75 mM phosphate buffer was added using the automatic dispenser. After incubation at 37 °C for 30 min, AAPH (153 mM) prepared freshly with 75 mM phosphate buffer was added to each well. The plate was agitated, and the decay of fluorescence was monitored continuously every 1 min for 60 min. The AUC of the oxidation of fluorescein was determined at wavelengths set in 485 nm for excitation and 528 nm for emission. The results were estimated using software Gen 5[™] (BioTek Instruments, Inc., Winooski, VT) and the ORAC activity was expressed as µmol of Trolox equivalents (TE) 100 g⁻¹ FW.

2.4 Statistical analyses

The results were expressed as mean of duplicate or triplicate according to the analysis and coefficient of

variation (CV) in percentage. Minimum and maximum values were also evaluated. The statistical analyses were determined using Infostat Software⁽¹⁸⁾. Variance analysis (ANOVA) and Tukey's test were performed to identified significant differences between species with a significance $p \le 0.05$. Correlation analysis between variables studied was carried out with the same statistical program.

3. Results and discussion

3.1 Bioactive compounds composition

The results for the determination of ascorbic acid and total phenolics compounds are in Table 2. Strawberry fruits (n = 10) contained the highest ascorbic acid concentration at 178.12 mg.100 g⁻¹ FW (p <0.0001), followed by native fruits (71.02 mg.100 g⁻¹ FW in average) and tomato (51.32 mg.100 g⁻¹ FW). On the other hand, onions, potatoes and sweet potatoes contained relatively low amount of ascorbic acid as compared to the fruits group. The mean concentrations obtained in this study were similar to those reported in bibliography⁽¹⁹⁾, although the data cannot be directly compared due to the incidence of genotypes, agroecological and cultural conditions, among others.

	Ascorbic acid mg ascorbic acid.100 g-1				Total phenolic content mg gallic acid.100 g ⁻¹				
	Mean ¹		CV (%)	min	max	Mean ¹	CV (%)	min	max
Arazá (red)	62.38	cd	10.78	55.63	70.86	128.56ª	9.59	111.35	139.35
Arazá (yellow)	69.17	bc	10.86	63.66	77.73	85.14 b	7.59	81.16	92.60
Guaviyú	73.20	bc	10.93	68.40	82.44	135.25 ª	9.99	119.93	145.53
Guayabo del país	79.31	b	22.48	55.87	105.09	62.21 °	27.29	41.74	87.29
Onion	5.26	е	19.42	3.26	6.54	11.46 d	8.99	9.29	12.57
Potato	10.24	е	36.11	5.19	15.68	15.20 d	20.14	8.48	18.62
Strawberry	178.12	а	7.21	156.30	196.74	87.08 b	8.49	78.64	104.80
Sweet Potato	5.73	е	31.09	2.92	8.10	2.56 d	39.43	1.53	4.45
Tomato	51.32	d	5.94	47.06	57.35	11.31 d	37.28	4.06	19.87

Table 2. Ascorbic acid and total phenolic compounds contents of fruits and vegetables analyzed

¹Mean as average and coefficient of variation (CV %) in each fruit and vegetable specie is presented. *min* and *max* correspond to minimum and maximum values determined, respectively. Values with different letters by the same column are significantly different (p < 0.05).

Table 2 also presents total phenolic content (TPC) in fruit or vegetable sample extracts. The highest TPC was found in guaviyú fruits (135.25 mg GAE.100 g⁻¹ FW) and arazá red (128.56 mg GAE.100 g⁻¹ FW) with p = 0.0001; followed by the others native fruits and strawberries. Arazá yellow and guayabo del país mean TPC values were 85.14 and 62.21 mg GAE.100 g⁻¹ FW, respectively. It is interesting to note that in all cases relatively high standard deviations in ascorbic acid and TPC values were found. Relatively high or low average ascorbic acid and total phenolic contents presented different coefficient of variation. Concentrations in arazá, guaviyú and strawberries genotypes result less variable than in the other fruit and vegetables analyzed. By contrast, in values of guayabo del país, potato and sweet potato high variability were found (CV >20%). In onion and tomato each parameter showed different variability. According to our sampling and analytical conditions, these differences could directly be due to genetic variations. Thus, some genotypes in each fruit or vegetable could be used as promising lines for improving ascorbic acid or total phenolic contents. Nevertheless. annual or productive cycles repetitions are needed to confirm these results.

Table 3 shows the mean anthocyanins, quercetin and carotenoids contents of fruits and vegetables found above the limit of quantitation. As expected, red/violet-colored fruits samples had significantly high total anthocyanins content. In guaviyú (n=3) and strawberry (n=10) fruits were estimated at 45.90 and 21.79 mg cyanidin-3-glucoside.100 g⁻¹ FW, respectively. These values are in accordance with those reported in literature for fruits and strawberries⁽²⁰⁾⁽²¹⁾.

Quercetin concentrations were determined only in onion extracts. The mean value was 17.51 as mg quercetin.100 g⁻¹ FW with a standard deviation of 4.88. These values are in accordance with those reported in bibliography⁽²²⁾. According to the high content found in onions and eating habits (average daily uptake to 10-100 mg quercetin)⁽²³⁾, it is easier to reach daily requirements. So, the selection of genotypes for improving quercetin content could be



useful in our country, as South America has the lowest levels of consumption of flavonoids⁽⁸⁾.

Table 3. Micronutrient content of fruits and vegetables analyzed

		,					
	Mean ¹	CV (%)	min	тах			
Anthocyanins (m	ıg cyanidi	n-3-glucos	side.100) g -1)			
Arazá (red)	8.13	6.90	7.62	8.93			
Arazá (yellow)	0.19	93.33	0.08	0.39			
Guaviyú	45.90	50.66	19.11	60.90			
Strawberry	21.79	22.63	15.75	26.38			
Quercetin (mg quercetin.100 g -1)							
Onion	17.51	27.89	6.84	23.72			
Carotenoids (mg β –carotene.100 g $^{-1}$)							
Potato	0.12	36.64	0.04	0.21			
Sweetpotato	5.07	91.79	0.55	11.23			
Tomato	4.70	8.90	4.30	5.28			

¹Mean as average and coefficient of variation in percentage (CV%) in each fruit and vegetable sample is presented. *min* and *max* correspond to minimum and maximum values determined, respectively.

Potato genotypes contained low concentrations of carotenoids (0.12 mg β -carotene.100g⁻¹), but differences between varieties were found. The mean carotenoids content determined as β carotene was higher in sweet potato genotypes with maximum value of 11.23 mg β -carotene.100 g⁻¹ FW. Sweet potatoes also presented high variability value (CV = 91.79%) due to the differences in composition that correspond with the color of flesh. The higher values found in varieties with yellow and orange color of flesh are in the range of green leafy vegetables and carrot concentrations, that are consider the mainly human diet intake of carotenoids and β -carotene⁽¹⁾⁽²⁰⁾. Although the mean carotenoids content in tomato samples is similar to that determined in sweet potato, the variability between varieties was lower. In addition

Agrociencia Uruguay 2021;25(NE2)



to the differences between the total content of carotenoids, these three species showed different carotenoids profiles⁽²⁴⁾. Potato, sweet potato and tomato varieties were predominantly constituted for lutein/zeaxanthin, β -carotene and lycopene, respectively. Therefore, a detailed profile characterization could improve the investigation about variability in genetic breeding.

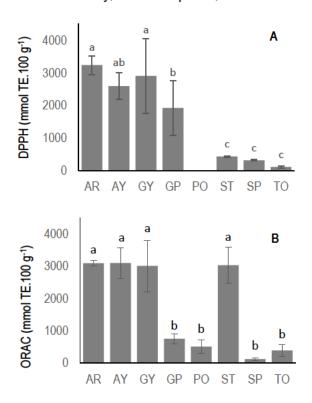
3.2 Antioxidant activities

Total antioxidant activity assays of fruits and vegetables are presented in Figure 1. The different species performed distinctly (p < 0.0001) and fruits had mainly the highest values in both analyses. These results are in accordance with other studies⁽²⁵⁾ and reinforce the fact that native fruits constitute an outstanding source of bioactive compounds that possess high antioxidant activity. The DPPH mean values were 3235.9, 2587.7, 2903.5 and 1919.1 µmol TE.100 g⁻¹ for arazá red, arazá yellow, guaviyú, and guayabo del país, respectively. Also, the values presented high variability in these fruit native species even when only three genotypes were analyzed in each. In strawberry (n=10), sweet potato (n=6) and tomato (n=19) genotypes the lower DPPH mean values were found. The variability between genotypes in these species was low.

The ORAC values of antioxidant extracts were represented in Figure 1B. The arazá red, arazá yellow, guaviyú and strawberry extracts showed the highest values with statistical significance (p<0.0001). The ORAC values were 3088.4, 3093.7, 3002.68 and 3026.0 μ mol TE.100 g-1, respectively.

It is interesting to note the antioxidative activity demonstrated in guayabo del país and strawberry extracts. The specificity and sensibility of each method provide different relative levels of activity. Hence, the combination of DPPH and ORAC assays supply a reliable assessment of the antioxidant properties of fruits and vegetables, but it is not possible to asseverate absolute values. Nevertheless, the values of relative antioxidant activity between genotypes of the same species and the contribution of specific antioxidant compounds could be achieved. Figure 1. Total antioxidant activity of fruit and vegetables measured by DPPH (A) and ORAC (B) as-

says. AR: Arazá (red); AY: Arazá (yellow); GY: Guaviyú; GP: Guayabo del país; PO: Potato; ST: Strawberry; SP: Sweetpotato; To: Tomato



Correlations between bioactive compounds studied and antioxidant capacities are presented in Table 4. Positive correlations between ascorbic acid, TPC, DPPH and ORAC were found. DPPH presented a positive correlation with TPC (n=55, r=0.75, p<0.0001), but there was no correlation with ascorbic acid (n=55, r=0.02, p=0.87). Probably due to the mechanism reaction, ascorbic acid is unsuitable for DPPH free radical scavenging and its bioactivity does not contribute with the total antioxidant activity. However, the significant correlation between ascorbic acid and ORAC (n=62, r=0.69, p<0.0001) suggests that in fruits and vegetables under our investigation ascorbic acid contributes to the antioxidant capacity determined by the ORAC assay.

High correlation between ORAC and TPC values were also found (r=0.88, p<0.0001). This strong correlation is in accordance also with other studies⁽²⁶⁾.

Table 4. Correlation relationship between variable	Table 4.	Correlation	relationship	between	variables
--	----------	-------------	--------------	---------	-----------

	Ascorbic acid	TPC ¹	DPPH	ORAC
Ascorbic acid	-	< 0.0001	0.87	< 0.0001
TPC	0.65	-	< 0.0001	< 0.0001
DPPH	0.02	0.75	-	< 0.0001
ORAC	0.69	0.88	0.51	-

¹ Total phenolic content. Represented as matrix r\p, with correlation coefficients\and the corresponding significance values indicated.

The results also indicated that antioxidant capacity varied depending on the assay applied. Furthermore, different analytical approaches are required to characterize the antioxidant capacity and the correlation with the lipophilic bioactive compounds, as carotenoids or tocopherols. Although their relatively low concentrations in fruit and vegetables found in this study, significant differences between genotypes in each species could affect the total potential antioxidant capacity. So, by eating a fruit and vegetable varied diet in accordance with the health recommendations, it is possible to access to nutraceutical compounds with different metabolic effects. Despite studies that have advanced details on the composition of foods, there are still many limitations in the literature on the ingestion of phenolic compounds. The studies do not use the same databases, many foods do not have their phenolic compounds identified, and there are differences in analytical methodologies. Further, it is remarkable that native fruits and some genotypes developed in INIA Breeding Programs have a high ranking on nutraceuticals and potential antioxidant capacity, indicating that each country needs to establish its own data for plant nutrients.

4. Conclusions

The analyses revealed a highly varied content of bioactive compounds and great health-promoting potential significantly depending on the fruit or vegetable analyzed. Additionally, the nutritional characterization of all tested selections or varieties of each cultivar turned out to be a useful tool to the genetic breeding programs and to elucidate the major contributor of antioxidant capacities, providing information for the consumers.



Author contribution statement

G.G., M.G., G.R., E.V., D.C. and F.I. conceived the research; V.F. and F.I. conducted the research and analyzed the data; E.V., D.C. and F.I reviewed and edited the manuscript; and V.F and F.I. wrote the paper.

References

1. National Institutes of Health. Vitamin C. Dietary Supplement Fact Sheets [Internet]. 2018 [cited 2021 Sep 22]. Available from: https://bit.ly/3EH4AMd.

2. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J Agric Food Chem. 2004;52(12):4026-37.

3. Ignat I, Volf I, Popa VI. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chem. 2011;126(4):1821-35.

4. Ponzo V, Goitre I, Fadda M, Gambino R, De Francesco A, Soldati L, Gentile L, Magistroni P, Cassader M, Bo S. Dietary flavonoid intake and cardiovascular risk: a population-based cohort study. J Transl Med [Internet]. 2015 [cited 2021 Sep 22];13(1):218. Available from: https://bit.ly/3kwtaaE.

5. Waheed Janabi AH, Kamboh AA, Saeed M, Xiaoyu L, BiBi J, Majeed F, Naveed M, Mughal MJ, Korejo NA, Kamboh R, Alagawany M, Lv H. Flavonoid-rich foods (FRF): a promising nutraceutical approach against lifespan-shortening diseases. Iran J Basic Med Sci [Internet]. 2020 [cited 2021 Sep 22];23(2):140-53. Available from: https://bit.ly/3u03aHP.

6. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res [Internet]. 2017 [cited 2021 Sep 22];61(1):1361779. Available from: https://bit.ly/3AxaiOl. 7. Black HS, Boehm F, Edge R, Truscott TG. The benefits and risks of certain dietary carotenoids that exhibit both anti- and pro-oxidative mechanisms: a comprehensive review. Antioxidants (Basel) [Internet]. 2020 [cited 2021 Sep 22];9(3):264. Available from: https://bit.ly/3AAAO9n.

8. Ballard CR, Maróstica MR. Health Benefits of Flavonoids. In: Campos MRS, editor. Bioactive Compounds: Health Benefits and Potential Applications. Duxford (GB): Woodhead Publishing; 2019. p. 185-201.

9. Colunga Biancatelli RML, Berrill M, Catravas JD, Marik PE. Quercetin and Vitamin C: an experimental, synergistic therapy for the prevention and treatment of SARS-CoV-2 related disease (COVID-19). Front Immunol [Internet]. 2020 [cited 2021 Sep 22];11:1451. Available from: https://bit.ly/2XGdwAx.

10. Saeedi-Boroujeni A, Mahmoudian-Sani MR. Anti-inflammatory potential of Quercetin in COVID-19 treatment. J Inflamm [Internet]. 2021 [cited 2021 Sep 22];18:3. Available from: https://bit.ly/3IPUQ9S.

11. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem. 2005;53(10):4290-302.

12. Ibanez F, Bang WY, Lombardini L, Cisneros-Zevallos L. Solving the controversy of healthier organic fruit: Leaf wounding triggers distant gene expression response of polyphenol biosynthesis in strawberry fruit (*Fragaria x ananassa*). Sci Rep [Internet]. 2019 [cited 2021 Sep 22];9:19239. Available from: https://bit.ly/3zBNNWV.

13. Giusti M, Wrolstad RE. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. Curr protoc food anal chem [Internet]. 2001 [cited 2021 Sep 22]:F1.2.1-13. Available from: https://bit.ly/2XTIGFU. 14. Ferrari VP, Ibáñez F, Cabrera D, Pintado B. Bioactive compounds and antioxidant capacity in native fruits of Uruguay. INNOTEC [Internet]. 2019 [cited 2021 Sep 22];19:64-75. Available from: https://bit.ly/3zyVJbs.

15. Pino MT, Domínguez DE, Saavedra J. Protocolos estandarizados para la valorización de frutos nativos del PROCISUR frente a la creciente demanda por ingredientes y aditivos especializados (Carotenoides, Antocianinas y Polifenoles). Santiago de Chile: IICA; 2018. 45p.

16. Held P. Performing Oxygen Radical Absorbance Capacity Assays with Synergy™ HT. BioTek [Internet]. 2006 [cited 2021 Sep 22]. 9p. Available from: https://bit.ly/39vjrLc.

17. Ou B, Huang D, Hampsch-Woodill M, Flanagan JA, Deemer EK. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. J Agric Food Chem. 2002;50(11):3122-8.

18. Di Rienzo JA, Casanoves F, Balzarini MG, González L, Tablada M, Robledo CW. InfoStat [Internet]. Version 2017. Córdoba: Universidad Nacional de Córdoba, Facultad de Ciencias Agropecuarias; 2017 [cited 2021 Sep 22]. Available from: https://bit.ly/3dDvlyu.

19. USDA. FoodData Central [Internet]. 2019 [cited 2021 Sep 22]. Available from: https://bit.ly/3nXW4Ta.

20. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem. 2006;54(11):4069-75.

21. Chaves VC, Calvete E, Reginatto FH. Quality properties and antioxidant activity of seven strawberry (Fragaria x ananassa duch) cultivars. Sci Hortic. 2017;225:293-8.



22. Rodrigues AS, Almeida DPF, Simal-Gándara J, Pérez-Gregorio MR. Onions: a source of flavonoids. In: Justino G, editor. Flavonoids: from biosynthesis to human health [Internet]. [place unknown]: IntechOpen; 2017 [cited 2021 Sep 22]. Available from: https://bit.ly/3u0faJ6.

23. Bischoff SC. Quercetin: potentials in the prevention and therapy of disease. Curr Opin Clin Nutr Metab Care. 2008;11(6):733-40.

24. Burns J, Fraser PD, Bramley PM. Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. Phytochemistry. 2003;62(6):939-47. 25. Proteggente AR, Pannala AS, Paganga G, Van Buren L, Wagner E, Wiseman S, Van De Put F, Dacombe C, Rice-Evans CA. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radic Res. 2002;36(2):217-33.

26. Cho Y-S, Yeum K-J, Chen C-Y, Beretta G, Tang G, Krinsky NI, Yoon S, Lee-Kim YC, Blumberg JB, Russell RM. Phytonutrients affecting hydrophilic and lipophilic antioxidant activities in fruits, vegetables and legumes. J Sci Food Agric. 2007;87(6):1096-107.