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Nutritional value, physicochemical characterization and bioactive properties of the Brazilian quinoa *BRS Piabiru*

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Chenopodium quinoa Willd. is an ancient food crop that has provided nutrition to Andean populations for thousands of years. *BRS Piabiru* is a quinoa genotype developed and adapted to tropical climate by the Brazilian Agricultural Research Corporation. In this work, *BRS Piabiru* quinoa was evaluated concerning its nutritional, physicochemical and phenolic composition and also its bioactive properties. This variety showed high carbohydrate and protein contents and a low-fat level, composed of 86% of unsaturated fatty acids, 60% of which are polyunsaturated fatty acids. Four organic acids were detected, with quinic acid as the most abundant, while α- and γ-tocopherols were the vitamin E isoforms found. Quercetin and kaempferol glycosides were the main phenolic compounds in the quinoa extract, which also revealed relevant antioxidant and antimicrobial activities, with no toxic effect. These results support the potential of *BRS Piabiru* quinoa as a nutritious food crop and a source of bioactive compounds.

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a grain-like food crop that has provided nutrition to Andean indigenous cultures for thousands of years.[1,2] Usually referred to as pseudo-cereal or pseudo-grain, the plant produces seeds that can be milled into flour, with technological features that resemble those of the Gramineae family, for instance wheat.[2,3] In addition to its excellent nutritional value, quinoa has been found to contain a high content of phytochemicals with positive health benefits, including phytosterols, phytoecdysteroids, phenolic compounds and bioactive peptides, which may contribute to metabolic, cardiovascular and gastrointestinal health.[2–4]

Quinoa has been classified as a "future smart food" by the Food and Agriculture Organization of the United Nations (FAO), *i.e.* "foods that can bolster dietary diversification, improve micronutrient intake, enhance soil health, require fewer inputs such as chemical fertilizers, and often prove resilient to climate changes and adverse farming conditions".[1] This pseudo-grain has the potential to enhance global food security for a growing world population, providing highly nutritious food that can be grown on lands not suitable for other crops.[5] Due to its relevance as an alternative food crop, quinoa has been promoted globally, with 2013 being declared the "International Year of Quinoa".[1,4] As its global popularity increases, quinoa cultivation has spread to more than 70 countries, including the United States, Canada, China, India, Finland, Australia, Kenya, the United Kingdom, Japan and Brazil.[1,4,6,7] Nevertheless, current major global producers remain the Andean countries Peru, Bolivia and Ecuador, respectively.[8]

Brazil is an established global grain supplier, and has placed the introduction of new crops into production systems as a high priority in research and development.[4] Agricultural diversification contributes to improve income, reduce costs, improve nutrient availability, protect the soil and reduce negative environmental impacts.[9] The Brazilian Agricultural Research Corporation (EMBRAPA) has been working for over twenty years in the selection and adaptation of quinoa varieties to be cultivated under Brazilian tropical climate.[4] *BRS Piabiru*, the first recommended quinoa for grain production in Brazil, is a selection of a breeding line originating from a plant population of Quito, Ecuador. The newly developed genotype was tested for years in variety trials, in Central Brazil, before

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being standardised for agronomic characteristics, such as rapid growth, tolerance to hydric stress, biomass production and nutrient cycle.[9,10] The Brazilian production of quinoa is expected to increase over the next years, reducing the pressure on Bolivia and Peru, where quinoa has become the sole grain crop export.[4]

Few studies have been performed on the chemical and technological aspects of the *BRS Piabiru* quinoa seeds. Palombini *et al.*[11] reported the fatty acid, proximate and amino acid compositions, antioxidant activity (inhibition of DPPH assay), total phenolic content (*Folin–Ciocalteu* reagent method), vitamin E and mineral contents. Nickel *et al.*[12] studied the variation in the total phenolic content, antioxidant capacity (DPPH and FRAP assays) and saponin content depending on the type of processing technology. Meneguetti *et al.*[13] reported the biological effects of the *BRS Piabiru* quinoa extract supplementation *in vivo* (rats), finding no hepatic nor renal toxicity. They also observed a decreased food intake, body weight, fat deposition, and blood triacylglycerol level in the supplemented groups. Moreover, three value added food applications applying the new quinoa variety have been reported to date: functional bread,[14] gluten-free granola[15] and gluten free cookie.[16]

In this work we perform detailed nutritional and physicochemical characterization of the Brazilian quinoa *BRS Piabiru*, and describe for the first time its phenolic compound profile and bioactivity using cell-based assays (antioxidant and antimicrobial activities and cytotoxicity).

2. Materials and methods

2.1 Quinoa samples

Samples of *Chenopodium quinoa* Willd. (*BRS Piabiru* genotype) were provided by the company Harmony Bioseeds, in partnership with EMBRAPA (Fig. 1). The quinoa plant was grown in the city of Chapada Gaúcha, Minas Gerais state, Brazil. Fresh seed samples were freeze dried (−49 °C, 0.08 bar, for 48 h, FreeZone 4.5 model 7750031, Labconco, Kansas, USA) and ground into a fine powder (20 mesh). The resulting powders were thoroughly mixed to obtain homogenized samples before analysis.

2.2 Chemical characterization

2.2.1 Nutritional and energetic value. The proximate composition was determined according to AOAC procedures as previously described.[17] The crude protein content was estimated by the macro-Kjeldahl method ($N \times 6.25$) using an automatic distillation and titration unit (model Pro-Nitro M Kjeldahl Steam Distillation System, Barcelona, Spain). Crude fat was determined by extracting the powdered sample with petroleum ether, using Soxhlet apparatus. Ash contents were determined by incineration at 550 ± 15 °C. Total carbohydrates were calculated by difference according to the following equation: (g per 100 g) = 100 − ($g_{fat} + g_{protein} + g_{ash}$).

2.2.2 Determination of free sugars. Free sugars were determined by HPLC as described by the authors[17] using chromatographic equipment provided with a refraction index detector (HPLC-RI; Knauer, Smartline system 1000, Berlin, Germany). The mobile phase consisted of an acetonitrile/water mixture (70 : 30 v/v). Separation was achieved in an Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 μm, Knauer) and quantification was performed by using melezitose as the internal standard. The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic) and expressed in g per 100 g of fresh weight (fw).

2.2.3 Determination of fatty acids. Fatty acids were determined by gas chromatography with a flame ionization detector (GC-FID), after the extraction and derivatization procedures previously described by the authors.[17] The analysis was carried

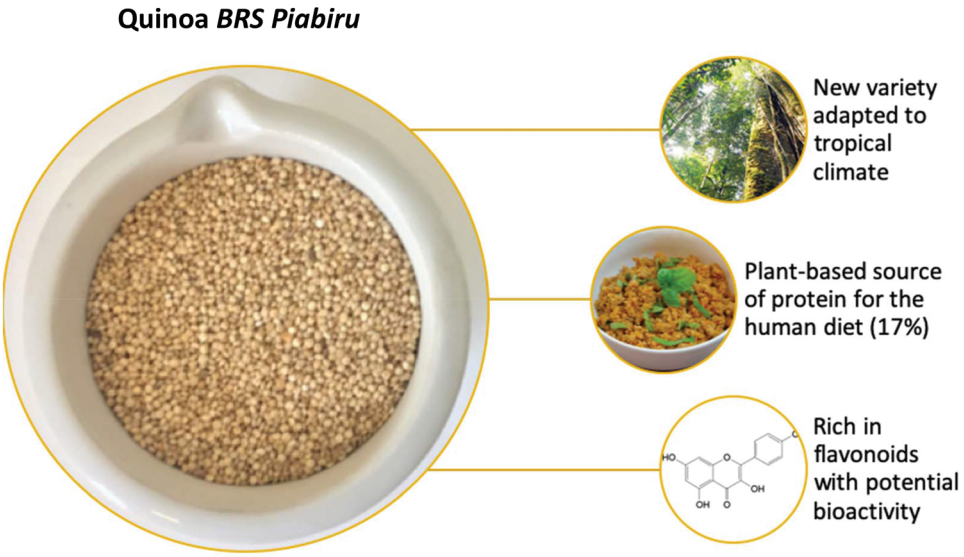

Fig. 1 Brazilian quinoa *BRS Piabiru* and some of its most important features.

out with a DANI model GC 1000 instrument and separation was achieved using a Zebron-Kame column (30 m × 0.25 mm ID × 0.20 μm *df*, Phenomenex, Lisbon, Portugal). FAMEs were identified by comparing their retention time with standards, and the results were processed using Clarity 4.0 software (DataApex, Podohradska, Czech Republic) and expressed in relative percentage of each fatty acid.

2.2.4 Determination of tocopherols. Tocopherols were determined on the freeze-dried samples (~500 mg) using a high performance liquid chromatography system coupled to a fluorescence detector (HPCL-FL; Knauer, Smartline system 1000, Berlin, Germany), as previously described.[18] The mobile phase consisted of a hexane/ethyl acetate mixture (70 : 30 v/v) and separation was achieved using a polyamide II column (4.6 × 250 mm, 5 μm, YMC Waters, Budapest, Hungary). The quantification of the different tocopherol isoforms (α-, β-, γ- and δ-) was performed based on calibration curves constructed using authentic standards (Sigma, St Louis, MO, USA) and by using the internal standard method (Tocol, Matreya, Pleasant Gap, PA, USA). The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic) and expressed in mg per 100 g of fw.

2.2.5 Determination of organic acids. Organic acids were determined by applying a previously described methodology and analysed using an ultra-fast liquid chromatograph coupled to a photodiode array detector.[17] The mobile phase consisted of sulphuric acid in water (3.6 mM) and separation was performed with a SphereClone reverse phase C18 column (4.6 × 250 mm, 5 μm, Phenomenex, Torrance, CA, USA). Quantification was carried out by comparison of the peak areas recorded at 215 and 240 nm, as preferred wavelengths, with calibration curves obtained from each standard compound. The results were processed using LabSolutions Multi LC-PDA software (Shimadzu Corporation, Kyoto, Japan) and expressed in g per 100 g of fresh weight (fw).

2.2.6 Determination of NaCl content. NaCl concentration was determined according to Mohr's method. Powdered samples (1 g) were dissolved in 20 mL of distilled water and filtered through Whatman No.4 paper five times. The pH of the final aqueous solution was then adjusted to 8.5 with sodium hydroxide, followed by the addition of 1 mL of potassium chromate solution (5%). The mixture was titrated against $AgNO_3$ (0.05 mol L^{-1}) until the appearance of the first reddish colour (Ag_2CrO_4 precipitate).[19] NaCl concentration was calculated using the following equation: salt content % = $[(V_{\text{titration of } AgNO_3} \times 0.00292)]/[(m_{\text{sample}})] \times 100$ (where 1 mL of $AgNO_3$ corresponds to 0.00292 g of NaCl). The results were expressed in g per 100 g of fresh weight (fw).

2.3 Physical characterization

2.3.1 pH assessment. pH was measured by blending 1 g of the powdered lyophilized sample with 20 mL of deionized water, according to the method described by Pellegrini *et al.*[20] Four measurements were undertaken using a calibrated digital pH meter (portable food and dairy pH meter HI 99161, Hanna Instruments, Woonsocket, RI, USA).

2.3.2 Colour measurement. A Minolta spectrophotometer (model CR-400; Konica Minolta Sensing, Inc., Japan) was used to measure the colour of the seeds. Using illuminant C and a diaphragm opening of 8 mm, the Hunter colour L^*, a^* and b^* values were measured through a computerized system using colour data software (Spectra Magic Nx, version CM-S100 W 2.03.0006, Konica Minolta company, Japan).

2.4 Determination of phenolic compounds and bioactivities

2.4.1 Hydroethanolic extract preparation. 30 mL of ethanol/water (80 : 20, v/v) was used to extract 1 g of freeze-dried sample. The extraction was performed twice in a magnetic stirrer plate (25 °C, 150 rpm, 1 h). The combined extracts were filtered (Whatman No. 4 paper) and vacuum-dried at 40 °C in a rotary evaporator (Büchi R-210, Flawil, Switzerland). The obtained aqueous extracts were frozen and freeze-dried.

2.4.2 Phenolic compounds. Phenolic compounds were determined by using an LC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA), following the method described by Gonçalves *et al.*[21] The freeze-dried extracts were dissolved in ethanol/water (80 : 20, v/v), to a concentration of 10 mg mL^{-1}. Detection was performed using a DAD (280, 330, and 370 nm as preferred wavelengths) and a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source working in negative mode. Identification of phenolic molecules was performed based on their retention time, UV-vis spectra and mass characteristics. Calibration curves for each phenolic standard (Extrasynthèse, Genay, France) were plotted based on the respective UV signal. The results were expressed as mg per g of extract.

2.4.3 Antioxidant activity. The antioxidant activity was assessed by two cell-based methods: (1) oxidative haemolysis inhibition assay (OxHLIA) and (2) inhibition of the production of thiobarbituric acid reactive substances (TBARS).

The OxHLIA assay was carried out as described in a previous study.[22] The results were expressed as delayed time of haemolysis (Δt), calculated as follows: Δt (min) = Ht_{50} (sample) − Ht_{50} (control), where Ht_{50} is the 50% haemolysis time (min) graphically obtained from the haemolysis curve of each sample concentration. The inhibitory concentrations of the extract able to promote a Δt haemolysis delay of 30 min ($IC_{50\ (60\ min)}$ μg mL^{-1}) and 60 min ($IC_{50\ (120\ min)}$, μg mL^{-1}) were calculated and expressed as IC_{50} values (mg mL^{-1} quinoa extract).

The method employed to measure the inhibition of production of thiobarbituric acid reactive substances (TBARS) by lipid peroxidation in brain homogenates was also described in a previous study.[23] The intensity of the pink colour resulting from the formation of the complex malondialdehyde–thiobarbituric acid (MDA-TBA) was measured at 532 nm. The inhibition ratios (%) were calculated and expressed as IC_{50} values (mg mL^{-1} quinoa extract).

2.4.4 Cytotoxicity evaluation. The quinoa extract was dissolved in water (4 mg mL^{-1}) and submitted to further dilutions. A cell culture (PLP2) was prepared using a freshly

harvested porcine liver according to the method previously described by Abreu *et al.*[24] The sulforhodamine B assay was performed to evaluate the extract hepatotoxic potential. Ellipticine (Sigma-Aldrich, St Louis, MO, USA) was employed as a positive control and the result was expressed as GI_{50} values, which correspond to the concentration of the extract that inhibited 50% of the net cell growth.

2.4.5 Antimicrobial activity. The antimicrobial activity was evaluated according to the procedure previously described by Corrêa *et al.*[25] Three Gram (+) and three Gram (−) bacteria were tested: *Bacillus cereus* (food isolate), *Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes* (NCTC 7973), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (human isolate) and *Salmonella typhimurium* (ATCC 13311), respectively. Additionally, six fungi were tested: *Aspergillus fumigatus* (ATCC 1022), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 36839) and *Penicillium verrucosum* var. cyclopium (food isolate). The results were expressed as the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of quinoa extracts.

2.5 Statistical analysis

Three samples of quinoa were used for each assay. All assays were carried out in triplicate ($n = 9$). The results were expressed as mean values with standard deviations (SD).

3. Results and discussion

3.1 Nutritional value and physicochemical composition

The nutritional value and physicochemical composition of *BRS Piabiru* quinoa are presented in Table 1. The seeds showed high protein content, with a mean concentration of 17.0 g per 100 g of fresh weight (fw), revealing a similar content to a previously published study on the same variety, 16.41 g per 100 g fw.[11] In a recent study, Pereira *et al.*[26] analysed thirty nine distinct genotypes of quinoa from Peru and Spain, with reported protein content ranging from 14.4 to 15.6 g per 100 g dw. These data highlight the outstanding potential of the studied Brazilian quinoa as a source of protein for the human diet, even when compared to other quinoa varieties. Considering other major grains, quinoa presents higher protein content than wheat (12.3%), maize (8.9%), rice (7.5%) and oat (16.1%).[2] Additional features include a balanced amino acid profile, easy digestibility and the absence of gluten, being its ingestion safe for celiac disease sufferers.[2,27] Quinoa protein profile has been compared to the milk protein, as it includes reasonable amounts of all the essential amino acids, being particularly rich in lysine.[28,29]

Table 1 Nutritional value (g per 100 g fw), energetic value (kcal per 100 g fw) and physicochemical composition of *BRS Piabiru* quinoa seeds (mean ± SD)

Nutritional value		Organic acids (g per 100 g fw)	
Moisture (%)	8.4 ± 0.1	Oxalic acid	0.603 ± 0.009
Proteins	17.0 ± 0.8	Quinic acid	3.6 ± 0.1
Lipids	6.0 ± 0.2	Malic acid	0.41 ± 0.03
Carbohydrates	66.5 ± 0.5	Fumaric acid	tr
Ash	2.1 ± 0.1	Total organic acids	4.6 ± 0.2
Energy (kcal per 100 g fw)	389 ± 1		
Free sugars (g per 100 g fw)		**Physicochemical variables**	
Fructose	0.060 ± 0.004	NaCl (g per 100 g fw)	0.39 ± 0.02
Glucose	0.31 ± 0.01	pH	5.65 ± 0.01
Sucrose	0.80 ± 0.03	Hunter scale colour parameters	
Total free sugars	1.17 ± 0.04	*L**	78.2 ± 0.4
		*a**	1.84 ± 0.08
		*b**	15.2 ± 0.1

tr – traces.

Carbohydrates appeared as the major macronutrient in the studied samples, accounting for 66.5 g per 100 g fw (Table 1). In general, the quinoa seed is characterized by a lower content of carbohydrates than cereals like wheat, barley, maize or rice, also presenting a low glycaemic index.[2,29]

Regarding free sugar composition, three distinct molecules were identified: two monosaccharides (fructose and glucose) and one disaccharide (sucrose) (Table 1). Sucrose was the most abundant one (0.80 g per 100 g fw), followed by glucose (0.31 g per 100 g fw) and fructose (0.06 g per 100 g fw). As a low-free sugar food crop, quinoa can be classified as part of a "low FODMAP diet", which has been shown to exert beneficial impacts on irritable bowel symptoms by limiting the ingestion of readily fermentable short-chain carbohydrates.[2]

Total fat reaches a mean concentration of 6.0 g per 100 g fw (Table 1), which is in agreement with the range of 2% to 10% described in the literature, considering distinct quinoa genotypes.[2,11,26] The fatty acid profile was composed of 85.97% of unsaturated fatty acids, 59.94% of which were polyunsaturated (PUFAs) (Table 2). The consumption of polyunsaturated fatty acids has been associated with a range of health benefits, for instance positive effects on cardiovascular disease, metabolism of prostaglandins, insulin sensitivity, the immune system and cell membrane function.[2,30] In the case of *BRS Piabiru* quinoa, the major PUFA was linoleic acid (C18:2n6, 56.70%), while monounsaturated fatty acids (MUFAs) were mostly represented by oleic acid (C18:1n9, 22.67%). The major saturated fatty acid found was palmitic (C16:0, 11.09%), which is also in agreement with the literature for other quinoa varieties.[30]

Quinoa is known for containing high concentrations of antioxidants, such as tocopherols, which act as scavengers of lipid peroxyl radicals.[11,30] In this study, α- and γ-tocopherols were the main isomers found in quinoa (Table 2). α-Tocopherol was found in a mean concentration of 0.919 mg per 100 g fw, which was slightly lower than the concentration of 1.16 mg per 100 g reported by Palombini *et al.*[11] for the same quinoa variety. Those authors reported a β + γ-tocopherol concentration of 1.08 mg per 100 g, which was less than half

Table 2 Composition of fatty acids (%) and tocopherols (mg per 100 g fw) of *BRS Piabiru* quinoa seeds (mean ± SD)

Fatty acid		Fatty acid	
C6:0	0.041 ± 0.004	C18:1n9	22.67 ± 0.04
C8:0	0.019 ± 0.001	C18:2n6	56.70 ± 0.03
C10:0	0.059 ± 0.001	C18:3n6	0.092 ± 0.004
C12:0	0.039 ± 0.001	C18:3n3	2.74 ± 0.01
C14:0	0.277 ± 0.001	C20:0	0.428 ± 0.004
C15:0	0.090 ± 0.003	C20:1	1.50 ± 0.08
C16:0	11.09 ± 0.01	C20:2	0.241 ± 0.002
C16:1	0.088 ± 0.001	C21:0	0.053 ± 0.001
C17:0	0.058 ± 0.001	C22:0	0.667 ± 0.007
C18:0	0.786 ± 0.004	C22:1	1.78 ± 0.01
SFA	14.03 ± 0.01	C22:2	0.172 ± 0.001
MUFA	26.04 ± 0.03	C23:0	0.073 ± 0.001
PUFA	59.94 ± 0.05	C24:0	0.35 ± 0.02
Tocopherols			
α-Tocopherol	0.919 ± 0.001		
γ-Tocopherol	2.67 ± 0.05		
Total tocopherols	3.59 ± 0.05		

Caproic acid (C6:0); caprylic acid (C8:0); capric acid (C10:0); lauric acid (C12:0); myristic acid (C14:0); pentadecanoic acid (C15:0); palmitic acid (C16:0); palmitoleic acid (C16:1); margaric acid (C17:0); stearic acid (C18:0); oleic acid (C18:1n9); linoleic acid (C18:2n6); γ-linolenic acid (C18:3n6); α-linolenic acid (C18:3n3); arachidic acid (C20:0); *cis*-11-eicosenoic acid (C20:1); eicosadienoic acid (C20:2); heneicosanoic acid (C21:0); behenic acid (C22:0); erucic acid (C22:1); docosadienoic acid (C22:2); tricosylic acid (C23:0); lignoceric acid (C24:0). SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

the concentration of γ-tocopherol found in the present study, 2.67 mg per 100 g. Despite considering the same variety, the discrepancies found in the tocopherol content between both studies may be due to distinct climatic and soil conditions, most probably associated with the specific regions in Brazil where the crops were grown.

Four organic acids were detected, quinic acid being the major one (3.6 g per 100 g fw), followed by oxalic (0.603 g per 100 g fw), malic (0.41 g per 100 g fw) and fumaric acids (trace amounts). Pereira *et al.*,[26] in a screening study on thirty-nine quinoa genotypes, also reported the presence of oxalic acid and, similarly, traces of fumaric acid; however, they did not detect quinic and malic acids. In a recent study performed by Heikkilä *et al.*[32] in an *in vivo* mice model, it was suggested that quinic acid could be helpful in the treatment of diabetes, by promoting insulin secretion from pancreatic beta cells.

The excess of sodium intake has been shown to be strongly associated with a plethora of health diseases.[33] This has stimulated the food industry to adopt interventions towards product development and reformulation to achieve a reduced final sodium content. The results for salt (NaCl) content in the *BRS Piabiru* quinoa showed a low mean value of 0.39 g per 100 g fw (Table 1). Considering this, the herein studied quinoa could be considered for the development of new processed food products that aim, and claim, reduced salt content.

The pH value (5.65) placed the studied quinoa grains as slightly more acidic than the six commercial genotypes analysed by Pellegrini *et al.*,[20] which presented a pH range of 6.42–6.63. Regarding colour, only whole grains were measured, before any milling process was employed; therefore, all measurements corresponded to the coloured outer layer of the grains (Fig. 1). The results showed that the grains were characterized by a high mean value of the parameter L^* (78.2), which indicates high luminosity. Additionally, the low value of the parameter a^* (1.84) indicates the absence of red-green intensities, and the parameter b^* value (15.2) is associated with a pale yellow colour. Escribano *et al.*[34] undertook an investigation of twenty-nine distinct varieties of quinoa, including white, black, yellow and red-violet genotypes. Blanca de Junín and Inia de Salcedo were two of the varieties analysed by these authors classified as "white quinoa", with results fairly similar to the ones found in this study, *i.e.*, L^* values of 73.97 and 75.60, a^* values of 1.54 and 1.53, and b^* values of 18.14 and 19.83, respectively.

Considering these results, the great nutritional value of the *BRS Piabiru* quinoa, along with its physicochemical features, makes this variety an attractive food crop for direct incorporation into the human diet and in a range of food formulations, especially considering its outstanding protein content and fatty acid composition.

3.2 Phenolic compounds and bioactive properties

The analysis of phenolic compounds in the Brazilian quinoa hydroethanolic extract was performed by HPLC-DAD-ESI/MS^n. Six flavonol glycosides were detected, derived from quercetin and kaempferol as concluded from their absorption and mass characteristics. Data are presented in Table 3 together with compound quantifications. Quercetin and kaempferol 3-*O*-rutinoside (peaks 5 and 6) were positively identified by comparison with commercial standards. Peaks 1, 2 and 3 were tentatively assigned as quercetin 3-*O*-(2″,6″-di-*O*-α-L-rhamnoside)-β-D-galactoside, quercetin 3-*O*-(2″-*O*-β-apioside-6″-*O*-α-rhamnoside)-β-galactoside and kaempferol 3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-galactoside, respectively, taking into account their previous description in quinoa samples.[7,37] The absorption and mass spectra of peak 4 were the same as peak 3, being tentatively associated with kaempferol 3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-glucoside considering that glucosides elute later than the corresponding galactosides.

More than 20 phenolic compounds have been described to date for distinct varieties of quinoa seeds, in either free or conjugated forms, liberated by alkaline, acid, and/or enzymatic hydrolysis.[3,7,31,36,37] The most abundant compounds reported are the flavonoids quercetin and kaempferol glycosides, as well as the phenolic acids vanillic acid, ferulic acid and their derivatives.[3,38] In this study, high concentrations of flavonol derivatives (quercetin and kaempferol) have been determined, which is in agreement with the literature; however, no significant amounts of phenolic acids were found. Considering that this is the first report on the identification of phenolic compounds in the quinoa *BRS Piabiru* genotype, this result might be explained by an actual low content, below our instrument detection limits, or inexistence of phenolic acids in this variety. Indeed, differences in phytochemical composition in

Table 3 Retention time (Rt), wavelengths of maximum absorption (λ_{max}), mass spectral data and quantification of the phenolic compounds tentatively identified in hydroethanolic extracts of *BRS Piabiru* quinoa seeds. The results are expressed as mean ± standard deviation, and the respective references for identification are presented

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification	Quantification (mg g^{-1})	References
1	14.37	352	755	760(38), 301(100)	Quercetin 3-*O*-(2″,6″-di-*O*-α-l-rhamnoside)-β-D-galactoside	93.5 ± 0.5	Gómez-Caravaca *et al.*[37] and Hirose *et al.*[7]
2	15.51	353	741	609(100), 301(80)	Quercetin 3-*O*-(2″-*O*-β-apioside-6″-*O*-α-rhamnoside)-β-galactoside	58.2 ± 0.3	Hirose *et al.*[7]
3	16.15	265 348	739	593(44), 285(100)	Kaempferol 3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-galactoside	23.73 ± 0.09	Gómez-Caravaca *et al.*[37] and Hirose *et al.*[7]
4	16.27	265 348	739	593(44), 285(100)	Kaempferol 3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-glucoside	21.5 ± 0.1	DAD, MS
5	17.53	353	609	301(100)	Quercetin 3-*O*-rutinoside	5.26 ± 0.09	Standard, Gómez-Caravaca *et al.*[37] and Tang, Li, Zhang *et al.*[31]
6	18.04	266 347	593	285(100)	Kaempferol 3-*O*-rutinoside	5.28 ± 0.06	Standard
					Total phenolic compounds	207.54 ± 0.2	

natural products can be explained by both genotypic or environmental factors.[2]

Phenolic compounds have been associated with a range of biological activities due to their effects on cell-signalling and metabolism, including antioxidant, anti-inflammatory, anticancer and cardioprotective effects.[2] The presence of these compounds in quinoa has been related to antidiabetic and anti-obesity properties, attributed to their α-glucosidase and pancreatic lipase inhibitory activities.[3]

The *in vitro* antioxidant, antibacterial and antifungal properties of the quinoa hydroethanolic extracts were assessed. Additionally, cytotoxicity of the extracts was also investigated. Several studies, including clinical trials, have demonstrated the antioxidant properties of quinoa, which have been attributed to its high polyphenol content.[2,3,29] Considering that antioxidant compounds act by distinct mechanisms, in this study we employed two different *in vitro* cell-based techniques to assess the antioxidant capacity of the *BRS Piabiru* quinoa hydroethanolic extract. The results of both assays are presented in Table 4.

Table 4 Antioxidant and cytotoxicity activities of *BRS Piabiru* quinoa seed hydroethanolic extracts (mean ± SD)

Cell-based antioxidant assays	
TBARS (EC50 μg mL^{-1})	764 ± 6
OxHLIA (IC_{50}, μg mL^{-1})	
Δt = 30 min	5.8 ± 0.2
Δt = 60 min	59 ± 1
Cytotoxicity (GI_{50} μg mL^{-1} values)	
PLP2	>400

EC_{50}: extract concentration corresponding to 50% of antioxidant activity. Trolox EC_{50} value: 23 μg mL^{-1} (TBARS inhibition). IC_{50} values are the concentration of the extract required to inhibit (delay) 50% of haemolysis for 30 min (IC_{50} (30 min)) and 60 min (IC_{50} (60 min)). Trolox IC_{50} values: 8.8 μg mL^{-1} (OxHLIA Δt = 30 min) and 19.6 μg mL^{-1} (OxHLIA Δt = 60 min). GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2. GI_{50} values for ellipticine (positive control): 3 μg mL^{-1} (PLP2).

The Brazilian quinoa extract was effective in diminishing the production of TBAR substances, which result from lipid peroxidation-induced oxidative stress. The extract presented a mean IC_{50} value of 764 μg mL^{-1}. A protective effect against lipid peroxidation was also observed in a human dietary intervention study conducted by Carvalho and Paya.[39] A double-blind intervention was conducted on 35 women with excess weight who consumed 25 grams of quinoa flakes daily for a period of four consecutive weeks. Their results showed a significant decrease in TBAR substances in the subjects' blood samples (3.06 to 2.89 μmol L^{-1}), which suggested the efficacy of quinoa intake as an antioxidant strategy for the human diet.

The antioxidant properties of the *BRS Piabiru* quinoa extracts were also confirmed by OxHLIA, a cell-based assay for evaluating the inhibition of free radical-induced haemolysis in sheep erythrocytes. A mean concentration of 5.8 μg mL^{-1} of the extract was found to inhibit the haemolysis by 50% for 30 minutes and 59 μg mL^{-1} for 60 minutes (Table 4). To the best of our knowledge, this was the first time that the OxHLIA assay was employed to evaluate the antioxidant properties of quinoa extracts. Other authors have also reported positive results for *in vitro*, non-cell-based, antioxidant assays of quinoa, for instance ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and DPPH radical scavenging assay.[11,20,34] Regarding the latter, only one study has been conducted on the same Brazilian quinoa variety investigated herein,[11] with positive although less expressive outcomes (IC_{50} average value of 313.25 μg mL^{-1} of the methanolic extract).

Several antinutritional factors have been identified by other authors in different varieties of quinoa, namely saponins, phytic acid, tannins, nitrates, oxalates, and trypsin inhibitors.[2,3,30] Nevertheless, the hydroethanolic extract studied herein did not present toxicity against the porcine liver primary culture PLP2, once its GI_{50} value was higher than the highest tested concentration (400 μg mL^{-1}) (Table 4). The employment of the porcine liver as an *in vitro* cytotoxicity model is justified by its similarity to the human liver, in terms of its cellular and physiological functioning.[25] The verified

Table 5 Antimicrobial activity of *BRS Piabiru* quinoa seed hydroethanolic extracts

Bacteria	Quinoa MIC/MBC	Streptomycin MIC/MBC	Ampicillin MIC/MBC
Staphylococcus aureus	1.0/2.0	0.04/0.1	0.25/0.45
Bacillus cereus	1.0/2.0	0.1/0.2	0.25/0.4
Listeria monocytogenes	1.0/2.0	0.2/0.3	0.4/0.5
Escherichia coli	1.0/2.0	0.2/0.3	0.4/0.5
Salmonella typhimurium	1.0/2.0	0.2/0.3	0.75/1.2
Enterobacter cloacae	1.0/2.0	0.2/0.3	0.25/0.5
Fungi	**Quinoa MIC/MFC**	**Ketoconazole MIC/MFC**	**Bifonazole MIC/MFC**
Aspergillus fumigatus	0.5/1.0	0.25/0.5	0.15/0.2
Aspergillus ochraceus	0.5/1.0	0.2/0.5	0.1/0.2
Aspergillus niger	1.0/2.0	0.2/0.5	0.15/0.2
Penicillium funiculosum	0.5/1.0	0.2/0.5	0.2/0.25
Penicillium ochrochloron	1.0/2.0	2.5/3.5	0.2/0.25
Penicillium verrucosum var. *cyclopium*	0.5/1.0	0.2/0.3	0.1/0.2

absence of cytotoxicity is of interest considering the potential use of the tested preparation for food and pharmaceutical formulations, confirming the safety of the Brazilian quinoa extract for incorporation in the human diet and other potential applications. Meneguetti *et al.*[13] investigated the biological effects of BRS Piabiru quinoa supplementation *in vivo*, in a rat diet study. Those authors found that the activities of the liver enzymes aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) did not change in the supplemented groups, with the values remaining at normal levels, confirming the absence of hepatotoxicity. Additionally, they observed decreased fat deposition and blood triacylglycerol levels in the supplemented groups.

The results for antimicrobial activity are shown in Table 5. The quinoa *BRS Piabiru* extract was tested against three Gram-positive and three Gram-negative bacteria, besides six fungi. The inhibitory and antibacterial activity displayed by the extract against both Gram-positive (*S. aureus*, *B. cereus* and *L. monocytogenes*) and Gram-negative (*E. coli*, *S. typhimurium* and *Enterobacter cloacae*) bacteria indicates the existence of an extensive antibiotic spectrum for their phytochemical constituents. The extract was equally active against all six tested bacteria. Besides, the quinoa extract was also effective against all six fungi tested, showing better results against *P. ochrochloron* than the fungicide ketoconazole.

The antibacterial activity of quinoa extracts was also observed by Miranda *et al.*[40] studying six different quinoa seeds, grown in three distinctive geographical zones of Chile. They prepared ethanolic extracts and studied their antibacterial activity *via* the disk diffusion assay technique. The extracts showed antimicrobial activity in the range of 8.3–14.8 mm inhibition zone for *E. coli* and 8.5–15.0 mm inhibition zone for *S. aureus*. Those authors also reported a positive correlation between the antimicrobial activity and the total phenolic content of the extracts. The presence of flavonoids in quinoa has also been previously linked to antimicrobial activity by other authors,[35] and may be responsible for the positive results found in the study herein.

4. Conclusion

The Brazilian quinoa *BRS Piabiru* presented a higher protein content than other previously described quinoa varieties, besides revealing a fatty acid composition with potential health benefits. Its outstanding nutritional value along with its physicochemical traits makes this food crop a suitable candidate for direct incorporation in the human diet. The quinoa variety also presented a high content of quercetin and kaempferol glycoside derivatives, which might be responsible for the positive results on the seeds' bioactivities. Overall, the results of the broad characterization carried out in this study suggest the viability of exploiting the Brazilian quinoa as a functional food, taking into account the verified *in vitro* antioxidant, antibacterial and antifungal activities, with the absence of cytotoxicity.

This work contributes to the growing debate on alternative sustainable and healthier foods by deriving information of a new, tropical climate adapted quinoa genotype. This information can potentially be used by the food and pharmaceutical industries in the development of new health-promoting products. This study also contributes to the FAO goal of turning quinoa into a commercial crop in Brazil, alleviating the increasing global demand pressure on Bolivia and Peru.

Conflicts of interest

The authors declare no conflict of interests.

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References

1 FAO, *Future Smart Food: Rediscovering hidden treasures of neglected and underutilized species for Zero Hunger in Asia*, Bangkok, 2018.
2 B. L. Graf, P. Rojas-Silva, L. E. Rojo, J. Delatorre-Herrera and M. E. Balde, (2015). Innovations in health value and functional food development of quinoa (*Chenopodium quinoa* Willd.), *Compr. Rev. Food Sci. Food Saf.*, 2015, **14**, 431–445.
3 R. Vilcacundo and B. Hernandez-Ledesma, Nutritional and biological value of quinoa (*Chenopodium quinoa* Willd.), *Curr. Opin. Food Sci.*, 2017, **14**, 1–6.
4 FAO & CIRAD, *State of the art report of quinoa around the world in 2013*, Rome, 2015.
5 D. E. Jarvis, Y. S. Ho, D. J. Lightfoot, S. M. Schmöckel, B. Li, T. J. A. Borm, H. Ohyanagi, K. Mineta, C. T. Michell, N. Saber, N. M. Kharbatia, R. R. Rupper, A. R. Sharp, N. Dally, B. A. Boughton, Y. H. Woo, G. Gao, E. G. W. M. Schijlen, X. Guo, A. A. Momin, S. Negrão, S. Al-Babili, C. Gehring, U. Roessner, C. Jung, K. Murphy, S. T. Arold, T. Gojobori, C. G. Van Der Linden, E. N. Van Loo, E. N. Jellen, P. J. Maughan and M. Tester, The genome of *Chenopodium quinoa*, *Nature*, 2017, **542**, 307–312.
6 B. Contreras-Jiménez, O. L. Torres-Vargas and M. E. Rodríguez-García, Physicochemical characterization of quinoa (*Chenopodium quinoa*) flour and isolated starch, *Food Chem.*, 2019, **298**, 124982.
7 Y. Hirose, T. Fujita, T. Ishii and N. Ueno, Antioxidative properties and flavonoid composition of *Chenopodium quinoa* seeds cultivated in Japan, *Food Chem.*, 2010, **119**, 1300–1306.
8 FAOSTAT, *Statistical Database*, 2019. Retrieved June 30, 2019, from http://www.fao.org/faostat/en/.
9 C. R. Spehar and R. L. de B. Santos, Quinoa BRS Piabiru: alternativa para diversificar os sistemas de produção de grãos, *Pesqui. Agropecu. Bras.*, 2001, **37**, 889–893.
10 C. R. Spehar and D. L. Teixeira, Quinoa (*Chenopodium quinoa*) reaction to herbicide residue in a Brazilian Savannah soil, *Pesqui. Agropecu. Bras.*, 2003, **38**, 771–776.
11 S. V. Palombini, T. Claus, S. A. Maruyama, A. K. Gohara, A. Henrique, P. Souza and M. Matsushita, Evaluation of nutritional compounds in new amaranth and quinoa cultivars, *Food Sci. Technol.*, 2013, **33**, 339–344.
12 J. Nickel, L. P. Spanier, F. T. Botelho, M. A. Gularte and E. Helbig, Effect of different types of processing on the total phenolic compound content, antioxidant capacity, and saponin content of *Chenopodium quinoa* Willd grains, *Food Chem.*, 2016, **209**, 139–143.
13 Q. A. Meneguetti, M. A. Brenzan, M. R. Batista, R. B. Bazotte, D. R. Silva and D. A. Garcia Cortez, Biological effects of hydrolyzed quinoa extract from seeds of *Chenopodium quinoa* Willd, *J. Med. Food*, 2011, **14**, 653–657.
14 V. Calderelli, M. D. T. Benassi and J. V. Visentainer, Quinoa and flaxseed: Potential ingredients in the production of bread with functional quality, *Braz. Arch. Biol. Technol.*, 2010, **53**, 981–986.
15 A. H. P. Souza, A. K. Gohara, L. M. Pagamunici, J. V. Visentainer, N. E. Souza and M. Matsushita, Development, characterization and chemometric analysis of gluten-free granolas containing whole flour of pseudocereals new cultivars, *Acta Sci., Technol.*, 2014, **36**, 157.
16 L. M. Pagamunici, A. K. Gohara, A. H. P. Souza, P. R. S. Bittencourt, A. S. Torquato, W. P. Batiston, S. T. M. Gomes, N. E. Souza, J. V. Visentainer and M. Matsushita, Using chemometric techniques to characterize gluten-free cookies containing the whole flour of a new quinoa cultivar, *J. Braz. Chem. Soc.*, 2014, **25**, 219–228.
17 L. Barros, E. Pereira, R. C. Calhelha, M. Dueñas, A. M. Carvalho, C. Santos-Buelga and I. C. F. R. Ferreira, Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of *Chenopodium ambrosioides* L, *J. Funct. Foods*, 2013, **5**, 1732–1740.
18 L. Barros, A. M. Carvalho, J. S. Morais and I. C. F. R. Ferreira, Strawberry-tree, blackthorn and rose fruits: Detailed characterisation in nutrients and phytochemicals with antioxidant properties, *Food Chem.*, 2010, **120**, 247–254.
19 T. M. Osaili, A. A. Al-Nabulsi, A. N. Olaimat, R. R. Shaker, M. Taha and R. A. Holley, Survival of *Escherichia coli*, O157: H7 during Manufacture and Storage of White Brined Cheese, *J. Food Sci.*, 2014, **79**, M1750–M1755.
20 M. Pellegrini, R. Lucas-gonzales, A. Ricci and J. Fontecha, Fatty acid, polyphenolic profile, techno-functional and antioxidant properties of flours obtained from quinoa (*Chenopodium quinoa* Willd) seeds, *Ind. Crops Prod.*, 2018, **111**, 38–46.
21 G. A. Gonçalves, R. C. G. Corrêa, L. Barros, M. I. Dias, R. C. Calhelha, V. G. Correa, A. Bracht, R. M. Peralta and I. C. F. R. Ferreira, Effects of in vitro gastrointestinal digestion and colonic fermentation on a rosemary (*Rosmarinus officinalis* L) extract rich in rosmarinic acid, *Food Chem.*, 2019, **271**, 393–400.
22 J. A. A. Garcia, R. C. G. Corrêa, L. Barros, C. Pereira, R. M. V. Abreu and I. C. F. R. Ferreira, Chemical composition and biological activities of Juçara (*Euterpe edulis* Martius) fruit by-products, a promising underexploited source of high-added value compounds, *J. Funct. Foods*, 2019, **55**, 325–332.

23 S. A. Petropoulos, Â. Fernandes, L. Barros, I. C. F. R. Ferreira and G. Ntatsi, Morphological, nutritional and chemical description of 'Vatikiotiko', an onion local landrace from Greece, *Food Chem.*, 2015, **182**, 156–163.

24 R. M. V. Abreu, I. C. F. R. Ferreira, R. C. Calhelha, R. T. Lima, M. H. Vasconcelos, F. Adega, R. Chaves and M. R. P. Queiroz, Anti-hepatocellular carcinoma activity using human HepG2 cells and carboxylate derivatives : In vitro evaluation, cell cycle analysis and QSAR studies, *Eur. J. Med. Chem.*, 2011, **46**, 5800–5806.

25 R. C. G. Corrêa, L. Barros, Â. Fernandes, M. Sokovic, R. M. Peralta, A. Brachtc and I. C. F. R. Ferreira, A natural food ingredient based on ergosterol: Optimization of the extraction from *Agaricus blazei*, evaluation of bioactive properties and incorporation in yogurts, *Food Funct.*, 2018, **9**(3), 1465–1474.

26 E. Pereira, C. Encina-zelada, L. Barros, U. Gonzales-barron, V. Cadavez and I. C. F. R. Ferreira, Chemical and nutritional characterization of *Chenopodium quinoa* Willd (quinoa) grains : A good alternative to nutritious food, *Food Chem.*, 2019, **280**, 110–114.

27 F. Janssen, A. Pauly, I. Rombouts, K. J. A. Jansens, L. J. Deleu and J. A. Delcour, Proteins of amaranth (*Amaranthus* spp.), buckwheat (*Fagopyrum* spp.), and quinoa (*Chenopodium* spp.): A food science and technology perspective, *Compr. Rev. Food Sci. Food Saf.*, 2017, **16**, 39–58.

28 A. Ceyhun Sezgin and N. Sanlier, A new generation plant for the conventional cuisine: Quinoa (*Chenopodium quinoa* Willd.), *Trends Food Sci. Technol.*, 2019, **86**, 51–58.

29 S. Dakhili, L. Abdolalizadeh, S. M. Hosseini, S. Shojaee-aliabadi and L. Mirmoghtadaie, Quinoa protein: Composition, structure and functional properties, *Food Chem.*, 2019, **299**, 125161.

30 A. M. Filho, M. R. Pirozi, J. T. Borges, H. M. Pinheiro Sant'Ana, J. B. Chaves and J. S. Coimbra, Quinoa: Nutritional, functional, and antinutritional aspects, *Crit. Rev. Food Sci. Nutr.*, 2017, **57**, 1618–1630.

31 Y. Tang, X. Li, P. X. Chen, B. Zhang, M. Hernandez, H. Zhang, M. F. Marcone, R. Liu and R. Tsao, Characterisation of phenolics, betanins and antioxidant activities in seeds of three *Chenopodium quino*a, Willd. genotypes, *Food Chem.*, 2015, **174**, 502–508.

32 E. Heikkilä, A. Hermant, J. Thevenet and J. S. Domingo, The plant product quinic acid activates Ca^{2+} - dependent mitochondrial function and promotes insulin secretion from pancreatic beta cells, *Br. J. Pharmacol.*, 2019, **176**, 3250–3263.

33 A. Allison and A. Fouladkhah, Adoptable interventions, human health, and food safety considerations for reducing sodium content of processed food products, *Foods*, 2018, **7**(2), 1–15.

34 J. Escribano, J. Cabanes, M. Jiménez-atiénzar, M. Ibañez-tremolada, L. R. Gómez-pando, F. García-carmona and F. Gandía-herrero, Characterization of betalains, saponins and antioxidant power in differently colored quinoa (*Chenopodium quinoa*) varieties, *Food Chem.*, 2017, **234**, 285–294.

35 Y. Tang, X. Li, B. Zhang, P. X. Chen, R. Liu and R. Tsao, Characterisation of phenolics, betanins and antioxidant activities in seeds of three *Chenopodium quino*a, Willd. genotypes, *Food Chem.*, 2015, **166**, 380–388.

36 S. Multari, A. Marsol-Vall, M. Keskitalo, B. Yang and J. P. Suomela, Effects of different drying temperatures on the content of phenolic compounds and carotenoids in quinoa seeds (*Chenopodium quinoa*) from Finland, *J. Food Compos. Anal.*, 2018, **72**, 75–82.

37 A. M. Gómez-Caravaca, A. Segura-Carretero, A. Fernández-Gutiérrez and M. F. Caboni, Simultaneous determination of phenolic compounds and saponins in quinoa (*Chenopodium quino*a, Willd) by a liquid chromatography-diode array detection-electrospray ionization-time-of-flight mass spectrometry methodology, *J. Agric. Food Chem.*, 2011, **59**, 10815–10825.

38 Y. Tang and R. Tsao, Phytochemicals in quinoa and amaranth grains and their antioxidant, anti-inflammatory, and potential health beneficial effects: a review, *Mol. Nutr. Food Res.*, 2017, **61**, 1600767.

39 G. De Carvalho and P. Paya, Metabolic parameters of postmenopausal women after quinoa or corn flakes intake – a prospective and double-blind study, *Int. J. Food Sci. Nutr.*, 2014, **7486**, 380–385.

40 M. Miranda, J. Delatorre-Herrera, A. Vega-gálvez, E. Jorquera, I. Quispe-fuentes and E. A. Martínez, Antimicrobial potential and phytochemical content of six diverse sources of quinoa seeds (*Chenopodium quinoa* Willd.), *Agric. Sci.*, 2014, **5**, 1015–1024.