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Original article

Oral administration of Costa Rican guava (*Psidium friedrichsthalianum*) juice induces changes in urinary excretion of energy-related compounds in Wistar rats determined by ^{1}H NMR

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ABSTRACT

Introduction: *Psidium friedrichsthalianum* fruits have *in vitro* antioxidant activity and their intake has been suggested to have potential health benefits; however, there is still no evidence on the potential *in vivo* effect of their consumption.
Objective: We aim to study the effect of the oral administration of a *P. friedrichsthalianum* juice on the urinary metabolome of adult male Wistar rats.
Methods: Acute and short-term interventions were carried out and urine sample metabolites were analyzed in a 600 MHz NMR instrument with a NOESY-presat sequence.and post-treatment effects were identified by comparison against control conditions.
Results: Acute administration caused a decrease in the excretion of citrate and allantoin in the 0 to 6 h period and citrate and α-ketoglutarate in the 6 to 12 h period. Lactate and Krebs cycle intermediates showed reduced excretion in the 12 to 24 h period. Short-term administration (for 7 days) decreased the excretion of Krebs cycle intermediates together with lactate and allantoin. The effect was stronger after short-term administration suggesting a dose-dependent effect.
Conclusion: *P. friedrichsthalianum* juice caused a change in rat excretion of energy metabolites, probably associated with an increase in aerobic metabolism, suggesting *in vivo* modulation of the redox balance.

1. Introduction

Epidemiological studies indicate that a high intake of fruits and vegetables correlates to a lower risk of developing chronic diseases [1,2], and a reduction in oxidative stress [3] and inflammation biomarkers [4]. Costa Rican guava (*Psidium friedrichsthalianum* is a *Myrtaceae* tree, naturally distributed from México to Panamá and Colombia that produces edible fruits with high organoleptic quality, which are commonly used for the production of juices [5] and fruit-based confectionaries [6].

P. friedrichsthalianum has been previously described as a source of compounds with *in vitro* antioxidant and anti-inflammatory activities in a model of pulmonary disease [6], therefore, there is an increased interest in the determination of the chemical composition of *P. friedrichsthalianum* fruits and their biological effects [7]. However, there is still limited information regarding the potential physiological effect of its consumption [5,6] in contrast to *Psidium guajava* (common guava), a botanically-related species highly studied and commonly used as a food

Abbreviations: NMR, Nuclear Magnetic Resonance; **^{1}H NMR**, ^{1}H (Proton) Nuclear Magnetic Resonance; **NOESY-presat**, Nuclear Overhauser Enhancement Spectroscopy pre-saturation.; **RPM**, revolutions per minute.; **BW**, Body weight mass.; **HMBC**, Heteronuclear Multiple Bond Correlation NMR; **HSQC**, Heteronuclear Single Quantum Coherence Spectroscopy; **DqCOSY**, Double Quantum Filtered Homonuclear Correlation Spectroscopy; **TE**, Trolox equivalents; **ORAC**, Oxygen Radical Absorbance Capacity

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source in several countries [8,9]. The common guava exhibits *in vitro* antioxidant activity [10] and has been suggested as a source of phytochemicals that may reduce glycemic, cholesterol and triglycerides levels [11].

P. friedrichsthalianum trees display a natural resistance to nematode-mediated root damage, a common challenge for commercial crops of *P. guajava* [12,13]. Then, due to agronomic advantages [12,13] and the potential role in health promotion suggested by *in vitro* analyses [6], we aimed to assess the *in vivo* biological effect of *P. friedrichsthalianum* fruits by examining the influence of its administration in the rat urinary metabolome, by using a ^{1}H NMR-based non-targeted approach.

The application of metabolomics to nutritional studies has been proposed as a valid approach to assess the high complexity of the interaction between an organism, its microbiome and the wide range of chemical compounds provided by the diet [14]. Urine is a valuable biofluid for metabolomic analysis since it can be collected in a non-invasive way [15,16] and requires minimal sample pre-treatment, which prevents changes in the metabolome [17]. Also, urine contains a wide diversity of metabolites (including xenobiotics) that are frequently present at higher concentrations, compared to blood or plasma [15]. Finally, as a concentrated excretion fluid, urine contains the phenotypical variations of different subjects [17]. Nuclear Magnetic Resonance (NMR) spectroscopy is considered a very valuable tool for nutritional assessment [18] by providing quantitative and reproducible measurements [19] using minimal sample pretreatment and generating databases with long term value [20].

Several pathologies are characterized by the accumulation of tissue alterations due to the presence of oxidative species because of unbalanced antioxidant regulatory systems [21,22]. Particularly, the regulation of elements involved in cellular energy metabolism has been reported to be affected by the mitochondrial redox status [23,24]. As a consequence, food phytochemicals that prevent the formation of cellular oxidative species, have been considered for the promotion of health and with several biological benefits [25].

It has been demonstrated that the administration of phytochemicals induces direct chemical scavenging activity of oxidative species and also regulates the expression of genes related to the antioxidant systems [25]. For instance, in biological systems, phytochemicals with *in vitro* antioxidant activity have been associated with the formation of reactive quinones, which interact with redox-sensitive species [25]. These molecules participate in the formation of protein adducts [26,27] and the promotion of cross-linking reactions between thiol groups [27], two mechanisms that regulate redox-based cell signaling pathways [28]. However, *in vivo* studies are required since it has been shown that the scavenging capacity of phytochemicals could be involved in the promotion of both, antioxidant and/or pro-oxidant states, in cellular systems [29].

Energy metabolism is a central element in cellular physiology and the evaluation of the role of the regulation of metabolic pathways and intermediaries involved in the energy supply of the cells has become a relevant topic in the investigation of the pathological processes of several diseases including cognitive disorders [30], cardiovascular diseases [31], diabetes [32] and tumorigenic processes [33].

This study was designed to provide some evidence of the potential effect of the administration of the antioxidant juice of this fruit, in the regulation of the metabolic balance of Wistar rats under normal food intake conditions.

2. Material and methods

2.1. Fruits and juice preparation

P. friedrischsthalianum fresh fruits were acquired from the National Center for Food Supply and Distribution in Costa Rica (PIMA-CENADA, Barreal, Heredia, Costa Rica). Fruits were originally harvested at Santiago de Puriscal, (San José, Costa Rica) in optimal ripening conditions, according to traditional agronomic practices. *P. friedrischsthalianum* fruits are traditionally consumed when naturally detach from the tree and when their color is from light green to yellow. Overripe fruits were eliminated by manual selection and the remaining fruits were washed and disinfected with a commercial solution of sodium hypochlorite for food industry applications (100 ppm/10 min).

Whole fruits were pulped through a 0.33 cm inner radius mesh using a semi-industrial pulper. The pulp was treated with a commercial pectinase kit (Pectinex® Ultra SP-L, Novozymes, Norway) at 37 °C (0.125 mL/kg fruit pulp) in a stainless-steel kettle to particle size reduction and release of potentially bioactive compounds. After 60 min of enzymatic treatment, the pulp was filtered through a synthetic cloth for fiber removal using a hydraulic semi-industrial press. The obtained juice was transferred to plastic sterile recipients and kept at −80 °C protected from light exposure using aluminum foil.

The content of humidity (AOAC 920.1512012), fiber (AOAC 985.292012), simple carbohydrates (HPLC-RID Zorbax carbohydrate column), organic acid (HPLC-DAD), total polyphenols (Folin-Ciocalteu spectrophotometric assay), total vitamin C (C18, Reverse Phase HPLC-UV), as well as the acidity (AOAC 942.152012), pH (AOAC 981.122012), Brix (AOAC 932.122012), and antioxidant capacity by ORAC test (Trolox equivalent activity measured by Fluorescence Spectroscopy), of the juice, were determined in a Certified External Independent Food Analysis Laboratory (CITA, Universidad de Costa Rica, San José, Costa Rica) according to validated methods (ISO-17025).

2.2. Animal model

Male, 52 days old Wistar rats (n = 8), obtained from the animal facility of Universidad de Costa Rica, were used. Animals included in the study were born on the same day from different mothers. Animal handling and all the experimental protocols were approved by the Institutional Committee for Use and Care of Laboratory Animals (CICUA, Universidad de Costa Rica, CICUA-024-015).

Animals were acclimated into the experimental location and housing equipment, 5 days prior to intervention. During the whole experimental period, including allocation time in metabolic cages, animals had free access to a commercial diet (20% humidity; 21% protein; 2% lipids; 21% starch; 72% total digestible fraction) and HCl-acidified tap water (pH = 2–3). Temperature (22.9 ± 1.0C) and relative humidity (73.1 ± 4.3%) in the room were constantly monitored during the study. Animals were located into social groups (groups of 4) in 90 × 40 cm plastic cages, except during urine collection, when individual rat metabolic cages (Tecniplast®) were used to allocate the animals. An automatized light/darkness cycle of 12 h (the light period from 6 a.m. to 6 p.m.) was used during the experimental protocol. Oral administration of samples (control and treatment conditions), for both acute and short-term administration, was performed using a metallic intra-gastric gavage (CRV FEEDING NDL 18GA 2IN 2/PK, Kent Scientific Corp, FNC-182) with a 5 mL sterile syringe. The administration was performed daily at 8:00 a.m. Before the location of the animals in metabolic cages, the remaining content in the bladder of animals was eliminated by a gentle abdominal compression.

Due to a problem with the water supply into the metabolic cage of one of the animals from the short-term administration experiment, its data was excluded from the analysis.

Animals were monitored for symptoms of stress and/or discomfort during social and isolated allocations and their body weights were also measured during the experiments as an indicator of good health. Post-Morten general examination of the organs and tissues was performed by the main author of the study by evaluation of the macroscopic appearance. These organs included skeletal muscle tissue, stomach, intestines, esophagus, heart, liver and kidneys.

2.3. Acute administration protocol

After the acclimatization period, animals (n = 8; BW 248.1 ± 12.8 g) were orally administered with 1.0 mL of acidified water (pH 2–3) and immediately located in single metabolic cages. Control urine samples were individually collected in three time periods (0 to 6; 6 to 12; and 12 to 24 h, post-treatment). Afterward, animals were kept 6 days in social allocation. At day seven, animals (n = 8; BW of 290.6 ± 14.7 g) were orally administered with 1.0 mL of *P. friedrischtalianum* juice (corresponding to a dose of 4 mL/kg BW) and immediately located in single metabolic cages. Urine samples from treated animals were individually collected in the same collection periods, as described previously.

2.4. Short-term administration protocol

Animals (n = 7; BW 266.3 ± 13.6 g) were placed individually in metabolic cages and control urine samples were collected during a 24 h period. Four days later, animals (n = 7; BW of 288.2 ± 14.1 g) were orally administered with 1.0 mL of *P. friedrischtalianum* juice (4 mL/kg BW), followed by daily doses of 0.5 mL of juice (2 mL/kg BW) during six consecutive days. After a washout period of 24 h to eliminate the acute effect of administration from the analysis, animals were placed individually in metabolic cages and urine samples were collected for 24 h from treated animals. Urine from each independent animal was collected as a single sample during the whole 24 h-period.

2.5. Sample preparation and ^1H NMR determination

Sample preparation was done according to previous protocols for metabolomics analyses of rat urine [34] with some modifications. Urine was centrifuged (14,000 RPM/ 5 min) to eliminate solid particles. Then, 1.0 mL of urine were mixed with 0.5 mL of a 0.25 M phosphate buffer (pH = 7.4; 20% D_2O; 1 mM TSP) containing 30 mM imidazole as internal pH indicator and sodium azide as preservative [35,36]. The buffer solution was prepared using Na_2HPO_4 (Sigma-Aldrich, Lot # BCBP7328V, Purity > 99,0%), anhydrous NaH_2PO_4 (Sigma-Aldrich, Lot # BCBP38101V), sodium trimethylsilylpropionate (TSP) (Sigma-Aldrich, Lot # MBBB 0475 V, 98% deuterated atoms), deuterated water (Sigma-Aldrich USA, Lot # MKBV 2445 V, 99.9% deuterated atoms), imidazole (Sigma-Aldrich, Purity > 99,0%), sodium azide (Sigma-Aldrich, Purity > 99,0%) and LC-MS grade distilled water (Agua Optima® UHPLC-UV, Fisher Scientific USA, Lot # 145439).

The urine-buffer mixture was then centrifuged (14,000 RPM/ 10 min) and 0.150 mL of the supernatant were transferred to a 1.7 mm OD NMR tube (Bruker 1.7 mm capillary tube, 103.5 mm length, 0.20 wall thickness, camber 60 μm). Samples were kept at 5 °C protected from light until analysis. Prior to analysis samples were left to warm up to 25 °C.

All NMR experiments were recorded at 300.8 Kelvin using a Bruker 600 MHz, Avance III (^1H frequency: 600.13 MHz), equipped with a 1.7 mm PATXI probe and running on Topspin 3.5pl7 (Bruker, Darmstadt, Germany). NOESY-presat (noesygppr1d) pulse sequence [37–39] was used with standardized and consistent pre-saturation protocols. Prior to measurements, temperature and 90° pulse were calibrated. Typically, 128 scans per sample were recorded using the following parameters: acquisition time was fixed to 3.389 s, relaxation delay D1 to 5.00 s and pulse width of 5.25 μs. The pre-saturation frequency was fixed to 4.685 ppm with a spectral width of 9590.8 Hz for a total acquisition size of 32 K data points and a spectral size of 64 K data points.

2.6. Spectral Processing

NMR spectra were analyzed using MestreNova 9.0.1 (MestreLab Research S.L., 2014) software. Each spectrum was manually handled using an exponential apodization of 0.3 Hz with 0.5 at the first point, which allowed a suitable balance of S/N ratio while retaining resolution and minimizing effects at the first point of the FID. A manual phase correction and an automatic correction of baseline (Whitaker softening) were also applied to each spectrum. After centering to TSP signal (δ = 0.00 ppm) a local alignment model was applied to all spectra excluding regions from δ = 8.1; to δ = 8.3 ppm and δ = 7.2 to δ = 7.3 ppm (Imidazole) due to its pH-dependent variation and regions from δ = 4.5 to δ = 6.0 ppm (water and urea signals) [38]. The rest of the spectrum (0.5 to 10.0 ppm) was divided into 0.02 ppm distance regions (*bins*). A tolerance level of 5% over the baseline and a normalization of the area under the curve using creatinine signal (δ = 4.05 ppm) as reference [40], was applied to the stalked spectrum. The normalization process was performed using the naturally occurring creatinine content of each sample.

In situ pH was determined during NMR measurement using imidazole signals as internal standard and calibrated with Chenomx NMR Suite 8.1 Software (Chemox Inc. Edmonton, Canada). The final pH in the NMR probe was determined by the specific chemical shift of imidazole signals (δ = 7.2 to δ = 7.3 ppm) for each sample. Identification of metabolites was performed by comparing suggested chemical structures from Chenomx NMR Suite 8.1 database with data from HMDB database [41]. Further confirmation was performed by ^1H; ^{13}C NMR and two-dimensional NMR approaches (HMBC, HSQC, dqCOSY) (Supplementary data; TopSpin 4.0.7 (Bruker, Darmstadt) was used to plot these spectra).

2.7. Statistical analysis

For each *bin* of the spectrum, the normalized area under the curve was compared under control and treatment conditions. The average change of the normalized area under the curve between treatments (for each *bin*) was considered as a selection criterion for the determination of signals associated with metabolomic responses to the treatment. Each *bin* was later associated with specific NMR signals in the spectra, and the normalized area under the curve, for each selected signal (singlet, doublet, triplet, multiplet), was determined individually for each sample.

For statistical analysis, the average value of the normalized area under the curve (for selected signals in treated and control group), were contrasted using a repeated-measures *t*-test with a significance value of 0.05. Logarithmic (Log) transformation to the Treatment/Control (T/C) area ratio was applied. A positive value in Log T/C was considered an increased urinary concentration of the metabolite, while a negative value was associated with a decreased urinary concentration, relative to control conditions. Concentration changes for discriminant signals were later confirmed with a second approach using a Chenomx NMR Suite 8.1 identification applying a *t*-test with a significance value of 0.05.

3. Results and discussion

3.1. General characteristics of the fruit samples

A composition profile and antioxidant activity of the fruits are shown in Table 1. Interestingly, compared to common guava (pH 4.3–4.5; total acidity of 0.3 to 0.5 g citric acid /100 g) [42], *P. friedrichsthalianum* fruits show stronger acidity, which explains why its traditional juice is prepared using a double dilution in water and is sweetened with sugar or a substitute. However, no dilution or sugar was added to the juice tested in this study to keep the administered volume low and to prevent the incorporation of an additional energy source different from the one already provided by the food or the juice itself.

3.2. General effects of the juice on the experimental animals

Post-*mortem* examination of the organs from experimental rats did

Table 1
Composition, antioxidant activity and some properties of *P. friedrichsthalianum* juice used for acute and short-term administration experiments.

Component		Reported value (mean ± SD)
Humidity (g/100 g)		89 ± 1
Fiber (g/100 g)		0.68 ± 0.04
Carbohydrates (g/100 g)	Glucose	0.68 ± 0.06
	Fructose	1.1 ± 0.2
	Sucrose	2.6 ± 0.1
Organic acids (g/100 g)	Citric acid	2.06 ± 0.05
	Malic acid	0.155 ± 0.003
	Succinic acid	1.41 ± 0.04
Acidity (g citric acid /100 g)		3.8 ± 0.1
pH		2.84 ± 0.01
Brix		10.490 ± 0.004
Vitamin C (mg/100 g)		29.4 ± 0.6
Total polyphenols (mg galic acid/100 g)		335 ± 7
ORAC (μmol TE/100 g)		24,316 ± 14

not show any visible changes in macro-anatomical structures (muscles, stomach, intestines, esophagus, heart, liver and kidneys) compared to untreated animals in preliminary tests. According to behavioral and physical daily monitoring and animal growing curves, there was also no evidence of toxicity or adverse effects induced by juice administration during the whole experiment. The expected reduction in activity during isolation periods [43,44] was observed, both under control and treated conditions.

Significant differences in urine volumes were found in the periods from 0 to 6 and 6 to 12 h of acute administration. As shown in Fig. 1A, higher urinary excretion in treated conditions was observed in the 0 to 6 h period (p = .042), whereas higher excretion in control conditions was obtained in the period from 6 to 12 h (p = .018). No significant differences were observed in the period from 12 to 24 h (p = .462) or the average excretion volume of the whole experiment (p = .497). Urinary volumes at the 24 h-period after short-time administration showed no significant differences between treated and control samples (p = .274) (Fig. 1B). The data suggest an acute effect of *P. friedrichsthalianum* administration on diuresis that should be further addressed.

The main metabolic pathways discussed in the present study are shown in Fig. 2. The interconnection of different metabolites will be discussed later in the text. The ^{1}H NMR spectrum of a typical rat urine sample is shown in Figs. 3 and 4 for up- and downfield regions respectively. The main characteristic of urine samples is the broad signal at δ = 5.80 ppm, corresponding to urea. As can be seen, the NOESY-presat sequence was very successful in suppressing the water signal, without significantly altering the regions on both sides of the irradiation. The addition of imidazole was useful to determine the pH of each sample (see Fig. 4), the variations of which would have gone unnoticed. For future experiments, it is advisable to adjust the pH using a microelectrode and adding base or acid as needed. Therefore, the use of phosphate buffer solution (pH 7.4) was not efficient to standardize the final pH of the samples for NMR determinations [34] causing undesirable shifts in the ^{1}H NMR spectra. On the other hand, addition to the prepared samples of a higher buffer proportion or any additional salty reagent would have resulted in poorer NMR measurements.

Although individual manual supervision of the alignment was performed on each spectrum, the local alignment model still reflected slight variations in chemical shifts and limited multivariate analysis. However, when comparing the averages of the corresponding *bins* of controls and treatments, a general trend was observed and each container was associated with specific signals and compounds, which were identified using Chenomx, the Human Metabolome Database and confirmed using 2D-NMR experiments. Since an NMR signal can be distributed in one or more *bins*, the assessment of significant variations was evaluated by comparing the normalized area under the curve in its respective spectrum. The results of this analysis are summarized in Tables 2, 3 and 4. For acute and short-term administration, no particular conclusion can be obtained from the variation in signals between the δ = 3.73–3.79 region, although they might be associated to carbohydrate-related components.

3.3. Acute administration scheme

Statistically significant variations in the urinary metabolome after acute administration of *P. friedrichsthalianum* juice are shown in Table 2. For the samples collected in the period from 0 to 6 h, a significant reduction in the intensity of ^{1}H NMR spectrum signals associated with citrate (p = .017 and 0.032) and allantoin (p = .006) and an increase in those related to taurine (p = .001), acetate (p = .036) and pantothenate (p = .001 and ≤ 0.001), was observed. For samples collected in the period from 6 to 12 h, a significant reduction in signals of citrate (p = .002), α-ketoglutarate (p < .001), and undertermined signals (p = .008), was observed. Additionally, a significant increase in urine concentration of taurine (p = .001), was detected. Samples comprising the period from 12 to 24 h showed a significant reduction in the signals corresponding to lactate (p = .004 and 0.002), acetate (p = .018), alanine (p = .050) and succinate (p = .011).

Then, the acute effect of the administration of *P. friedrichsthalianum* juice mainly caused a reduction in urinary excretion of mediators of the energetic pathways (citrate, α-ketoglutarate and lactate) and allantoin, and a higher excretion of taurine. A reduction in the excretion of the intermediates of the Krebs cycle could suggest the utilization of these compounds in the oxidative energetic cell pathways (Fig. 2) and there is *in vitro* evidence of the regulation of oxidative metabolic processes by *P. friedrichsthalianum* fruit [6] and common guava [8]. In the specific case

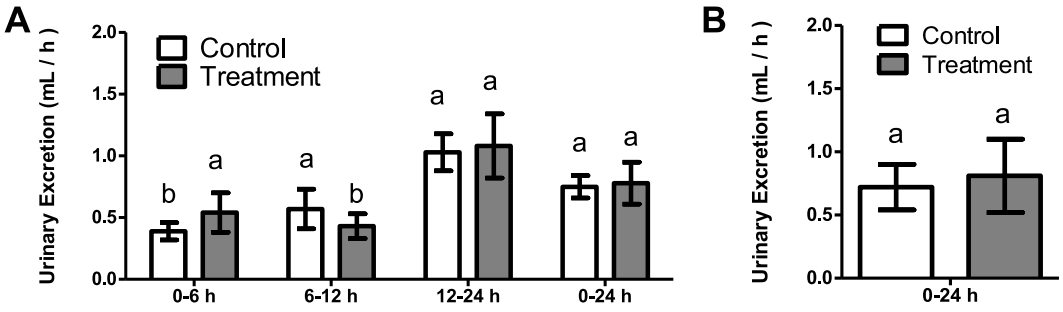

Fig. 1. Urinary excretion volumes of Wistar rats (Mean ± SD) after administration of *P. friedrichsthalianum* juice in (A) acute and (B) short-term schemes. Statistical analysis corresponds to a paired *t*-test comparison between control and experimental treatment conditions at the same collection time. Bars showing different letters correspond to statistical significance using an α of 0.05. (A) Higher urinary excretion in treated conditions was observed in the 0 to 6 h-period whereas higher urinary excretion in control conditions was obtained in the 6 to 12 h period (B) 24- after short -term administration showed no significant differences between treated and control samples.

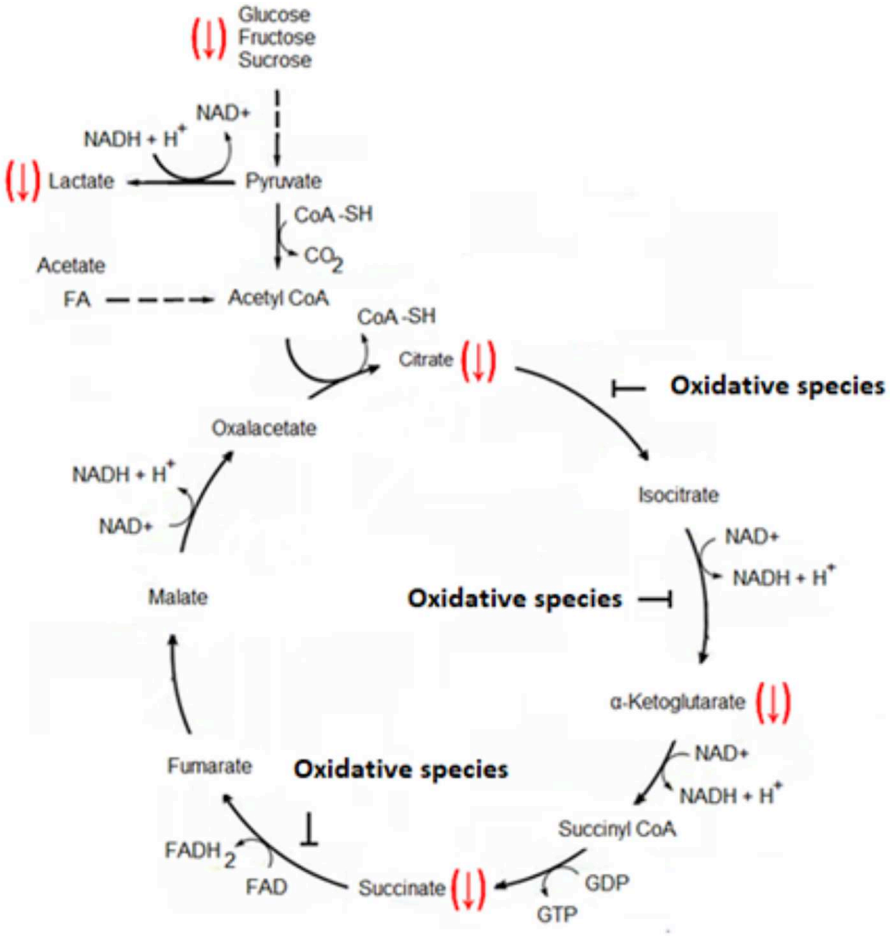

Fig. 2. Krebs cycle and oxidative metabolism of energetic intermediates. Red arrows indicate a significant reduction in urinary metabolite concentration in Wistar rats after a short-term administration of *P. friedrichsthalianum* juice according to metabolomic data. Oral administration of *P. friedrichsthalianum* juice in a Wistar rat model causes changes in urine energetic intermediates, consistent with an in vivo increase in aerobic metabolism and antioxidant effects. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of citrate, its excretion has been related to changes in urinary pH [45] and animal age [46] but no significant effect of those variables was considered to be related to our results since no significant differences in urinary pH between control and treated conditions were observed and same-age animals were used in these experiments.

In agreement with our study, human ingestion of a polyphenol-rich extract from *Hibiscus sabdariffa* fruit calyces has been related to a reduction in urinary excretion of some Krebs cycle intermediates (fumarate and malate) at the 3 h post-administration period [47]. On the other hand, it has been suggested that administration of quercetin is related with increased urinary excretion of Krebs cycle intermediates (citrate, α-ketoglutarate and succinate) during the first 12 h after the intervention, but with a reduced comparative urinary excretion during the period from 12 to 24 h after treatment. Quercetin administration is associated with reduced excretion of lactate [48] in concordance with results obtained with *P.friedrichsthalianum* juice in this acute intervention scheme. In agreement with our study, acute administration of resveratrol in a rat model has been related to reduced urinary excretion of α-ketoglutarate, nevertheless, the authors reported also a reduction in pantothenate excretion at a 24 h-collection period [49].

Pantothenate is a vitamin utilized for the synthesis of Coenzyme A, an essential cofactor in several metabolic reactions [50,51] such as the synthesis of Acetyl-CoA [52], the initial metabolite for the Krebs cycle in the process of oxidation of carbohydrates and/or fatty acids. Synthesis of Coenzyme A from pantothenate involves the activity of different isoforms of pantothenate kinases (PANK) [53–55]. The activity of relevant isoforms of PANK has been reported to be modulated by both endogenous intermediary metabolites involved in energetic pathways [55–57] but also by xenobiotic compounds [54]. Plasma concentrations of pantothenate are reported to be increased in patients with deficient PANK2 activity [53], therefore reduced synthesis of acetyl CoA could be related to higher plasmatic levels and consequently urinary excretion of pantothenate in animal models. Then, an increase in pantothenate excretion after administration of *P. friedrichsthalianum* juice suggests also a potential effect on intermediary metabolism that should be further analyzed.

Even when traditionally, lactate has been described as the cytosolic end product of glycolysis in environments of low oxygen availability [58], there is also some evidence of aerobic oxidation of lactate in the mitochondrion in the presence of oxygen or when the respiratory chain is highly active [59]. Then, blood lactate accumulation could be the result of inhibition of the Krebs cycle and its absence could indicate aerobic metabolism is increased. The reduction in lactate excretion after acute administration of *P. friedrichsthalianum* juice would agree with the promotion of *in vivo* oxidative-mediated processes.

Allantoin is an oxidative product of ureate [60], whose non-enzymatic transformation has been suggested as a biomarker of *in vivo* oxidative distress [61]. Then, a reduction in allantoin excretion after administration of *P. friedrichsthalianum* juice suggests also a potential acute effect on the *in vivo* oxidative status, which correlates with the behavior observed for lactate and Krebs intermediates. In rodents, allantoin synthesis could be mediated by the action of the enzyme uricase

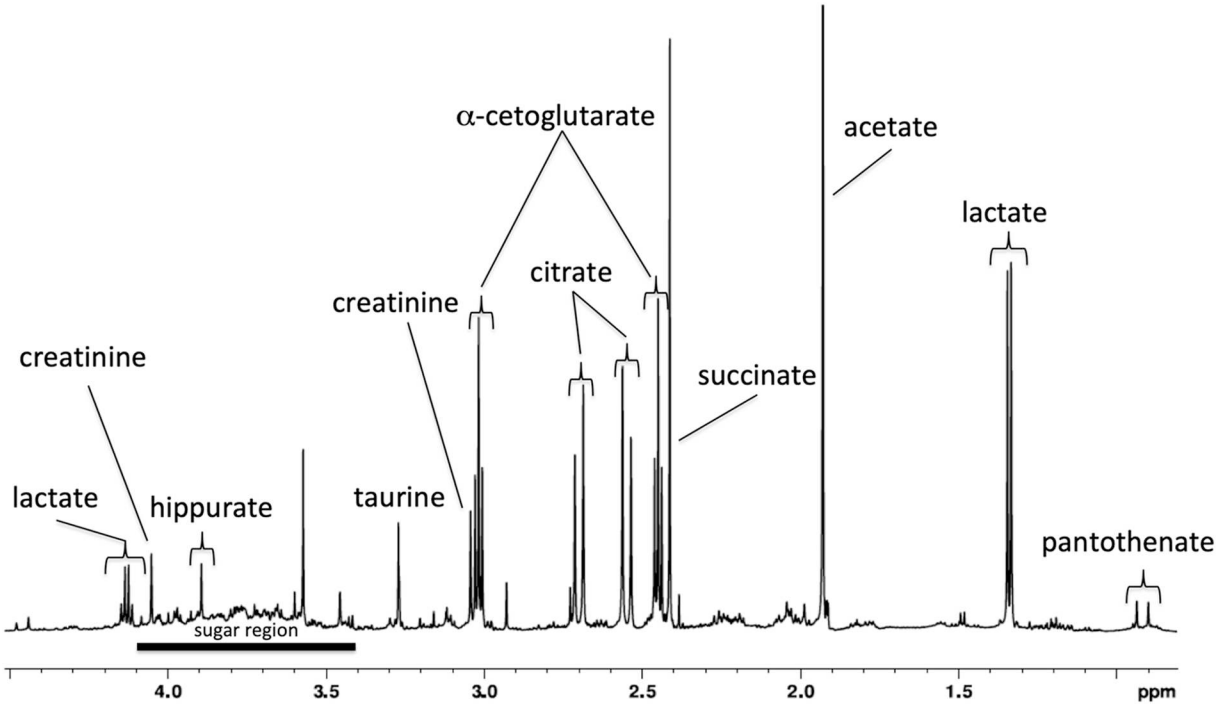

Fig. 3. ^{1}H NMR spectrum (600 MHz, Bruker H_2O:D_2O 8:2) from a Wistar rat urine sample (0.1 mL of sample in 0.05 mL of 0.25 M phosphate buffer containing 1 mM TSP, 30 mM imidazole and 3 mM of sodium azide) for the determination of the acute and short-term effect of *P. friedrichsthalianum* juice administration (aliphatic region δ = 0.80 to 4.50 ppm shown).

[62], nevertheless, no significant variation of this variable is expected for this experiment, even when the promotion of uricase expression has been related to glucocorticoid activity and may be linked to housing stress [63].

Allocation in metabolic cages could induce moderate stress leading to some glucocorticoid secretion [64], but it was not considered a significant factor, having that an increase in allantoin excretion (instead of a reduction), would be expected associated to an increase in the allocation time. Allantoin excretion has also been related to protein synthesis and intake [65] but these factors are not taken into consideration in this study due to the use of (a) a standardized diet during the whole experiment and (b) a short-lasting model, where no

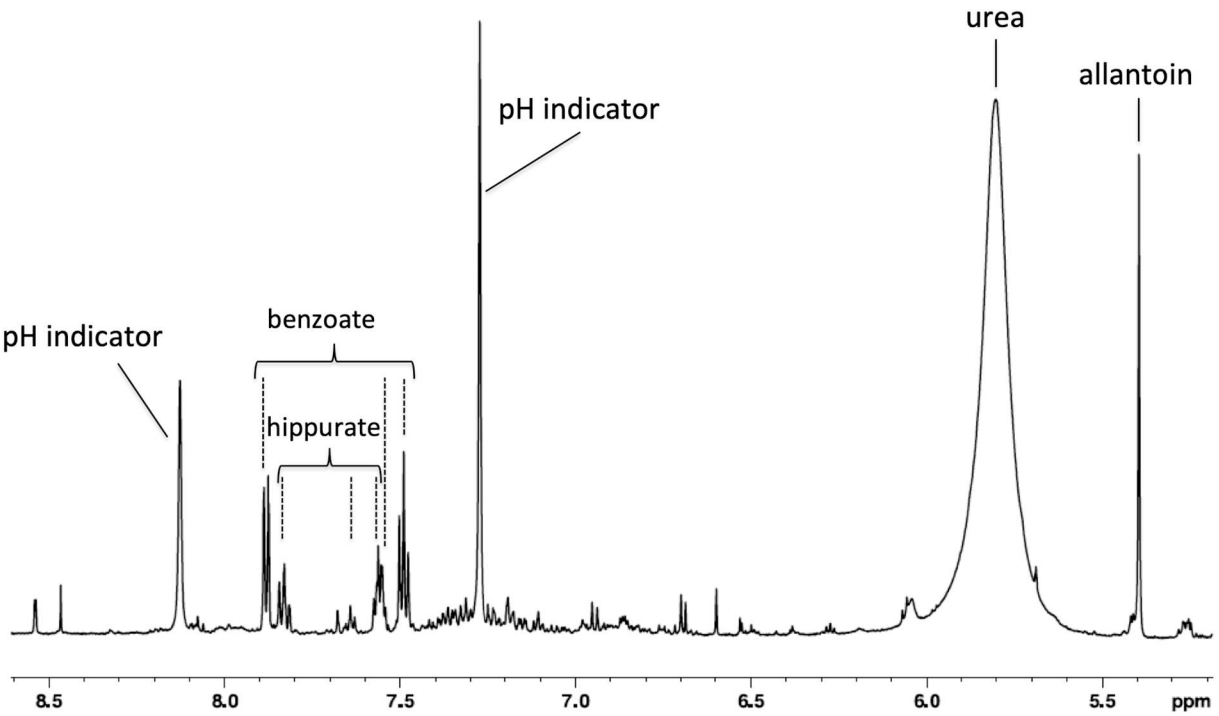

Fig. 4. ^{1}H NMR spectrum (600 MHz, Bruker H_2O:D_2O 8:2) from a Wistar rat urine sample (0.1 mL of sample in 0.05 mL of 0.25 M phosphate buffer containing 1 mM TSP, 30 mM imidazole and 3 mM of sodium azide) for the determination of the acute and short-term effect of *P. friedrichsthalianum* juice administration (downfield δ = 5.20 to 8.60 ppm shown).

Table 2
Urine metabolomic changes determined after an acute administration of *P. friedrichsthalianum* juice in Wistar rats (Integration was done using MestreNova software).

Collection Period	Metabolite	δ (ppm)	*P* value	Log T/C
0–6 h	Pantothenate	d(0.90)	.001	0.22 (↑)
		d(0.94)	< .001	0.30 (↑)
	Acetate	s(1.93)	.036	0.11 (↑)
	Citrate	d(2.71)	.017	−0.08 (↓)
		d(2.56)	.032	−0.06 (↓)
	α-ketoglutarate	t(2.45)	.142	−0.04 (↓)
		t(3.02)	.076	−0.05 (↓)
	Taurine	m(3.41)	.001	0.11 (↑)
	Allantoin	s(6.06)	.006	−0.18 (↓)
6–12 h	Citrate	d(2.56)	.002	−0.12 (↓)
		d(2.71)	.002	−0.13 (↓)
	α-ketoglutarate	t(2.45)	< .001	−0.09 (↓)
		t(3.02)	< .001	−0.12 (↓)
	Taurine	m(3.41)	.001	0.14 (↑)
	Undetermined	3.73–3.79	.008	−0.11 (↓)
12–24 h	Lactate	d (1.34)	.004	−0.45 (↓)
		d(4.15)	.002	−0.36 (↓)
	Alanine	d(1.49)	.050	−0.42 (↓)
	Acetate	s (1.93)	.018	−0.56 (↓)
	Succinate	s(2.42)	.011	−0.29 (↓)

δ = Chemical shift in ppm (parts per million by frequency); **s** = singlet; **d** = doublet; **t** = triplet; **m** = multiplet; **T/C** = Treatment Area/Control Area ratio; **(↑)** = increased excretion; **(↓)** = decreased excretion; **p** = associated probability from repeated measurements t-test.

Table 3
Urine metabolomic changes determined after a short-term administration (for 7 days) of *P. friedrichsthalianum* juice in Wistar rats (Integration was done using MestreNova software).

Metabolite	δ (ppm)	P value	Log T/C
α-ketoglutarate	t (2.45)	.001	−0.12 (↓)
	t (3.01)	.001	−0.12 (↓)
Citrate	d (2.55)	< .001	−0.14 (↓)
	d (2.70)	< .001	−0.14 (↓)
Glycine	s (3.57)	.001	−0.32 (↓)
Succinate	s (2.41)	.001	−0.16 (↓)
Lactate	d (1.34)	.035	−0.46 (↓)
Undetermined	d (3.78)	.001	−0.12 (↓)
Allantoin	s (5.40)	.002	−0.07 (↓)
Benzoate	t (7.49)	.004	−0.47 (↓)
	d (7.88)	.009	−0.53 (↓)
Hippurate	d (7.84)	.046	0.25 (↑)

δ = Chemical shift in ppm (parts per million by frequency); **s** = singlet; **d** = doublet; **t** = triplet; **m** = multiplet; **T/C** = Treatment Area/Control Area ratio; **(↑)** = increased excretion; **(↓)** = decreased excretion; **p** = associated probability from repeated measurements t-test.

Table 4
Calculated concentration ratio (treatment/control) for creatinine-normalized excretion of urinary metabolites after a short-term administration of *P. friedrichsthalianum* in Wistar rats (Integration was done using Chenomx software).

Metabolite	δ (ppm)	P	Ratio T/C
Lactate	d(1.34)	.05	0.38 (↓)
Glycine	s(3.55)	.02	0.38 (↓)
Benzoate	t(7.49)	.02	0.35 (↓)
Hippurate	t(7.63)	.03	1.81 (↑)

δ = Chemical shift in ppm (parts per million by frequency); **s** = singlet; **d** = doublet; **t** = triplet; **m** = multiplet; **T/C** = Treatment Area/Control Area ratio; **(↑)** = increased excretion; **(↓)** = decreased excretion; **p** = associated probability from repeated measurements t-test.

significant changes in protein synthesis should be expected.

After *P. friedrichsthalianum* juice acute administration, acetate excretion shows a dual effect, increasing during the 0 to 6 h period and with a reduction in the late 12 to 24 h period, when compared to control conditions. Acetate is a plasma normal circulating compound [66] resulting from intestinal microbial metabolism of dietary compounds [67]. An increase in acetate concentration in plasma has been shown as a consequence of decreased aerobic oxidation in the mitochondria [67] and has been associated with an increase of plasma glucose in clinical diabetes [68]. Also, the utilization of plasma acetate by the brain has been reported in aerobic metabolic conditions [69]. In this scenario, the reduction of its excretion in the latest periods would suggest also the promotion of oxidative metabolism, while increasing in the first 6 h post-administration would represent the opposite effect.

3.4. Short-term administration scheme

Statistically significant variations in the urinary metabolome after short-term administration of *P. friedrichsthalianum* juice are shown in Table 3. *P. friedrichsthalianum* induced a significant reduction in ^{1}H NMR signals associated with Krebs cycle intermediates (citrate ($p < .001$), succinate ($p = .001$), and α-ketoglutarate (p = .001)), together with lactate ($p = .035$), allantoin ($p = .002$), glycine (p = .001), benzoate ($p = .004$ and 0.009), and undetermined signals in the characteristic region of carbohydrates ($p = .001$). Also, a significant increase in urinary excretion of hippurate ($p = .046$) was observed after this intervention. In addition, Table 4 shows the change in ratios (treatment/control) for the creatinine-normalized concentration of urine metabolites by a second targeted approach, calculated using Chenomx Software. A significant reduction in lactate ($p = .05$), glycine ($p = .02$) and benzoate (p = .02) excretion was confirmed, whereas an increase in urine concentration of hippurate (p = .03) was observed as the result of this calculation.

An eventual reduction in the urinary excretion of carbohydrates could be related to the promotion of energetic metabolism but also with a hypoglycemic effect in the animals during the experiment. Although there is no report of an effect of *P.friedrichsthalianum* juice in animals or due to human consumption on the glycemic values, the administration of the botanical related species *P. guajava* has been associated with a hypoglycemic effect [11].

As described previously, a reduction in the area of signals of Krebs cycle compounds [23,24], lactate [58,59] and allantoin [61] together with the observed reduction in signals among the region of the sugars, suggests a decrease in the excretion of energetic intermediates. This reduction could be a potential promotion of aerobic metabolism induced by the combination of *P. friedrichsthalianum* phytochemicals.

Reduction in allantoin excretion was observed when rats were administered with curcumin extract from *Curcuma longa* for 25 days [70]. In a similar animal model, this plant induced a reduction in 2-oxoglutarate (α-ketoglutarate) urinary excretion [71]. A 35 days-period intervention with cranberry in rat models, showed an increase in urinary citrate excretion [72]. As in the case of *P. friedrichsthalianum* fruits, *C. longa* [73] and cranberries [74] have been described as foods with *in vitro* antioxidant activity.

Several phytochemicals have been involved in the modulation of the redox balance of cells by inducing direct scavenging activity or modulation of signaling pathways [25]. Therefore, the observed variations in the excretion of metabolites in the acute intervention with *P. friedrichsthalianum* juice suggest a possible fast inhibitory effect on certain enzymes and/or mediated by direct scavenging activity. On the other hand, the more stable patterns obtained during the short-term intervention scheme are probably related to an effect on the modulation of gene expression of endogenous regulatory systems such as those mediated by antioxidant enzymes. Also, a cellular response to phytochemical byproducts is possible and could explain the effects observed in these experiments [28].

P. friedrichsthalianum juice analysis showed *in vitro* antioxidant activity of 243.2 μmol TE/g (Table 1), which seems low compared to the values reported for other sources of dietary antioxidants, such as *Camellia sinensis* green tea (13,609.7 ± 1301.3 μmol TE/g) [75], *Eucalyptus globulus* leaves (284,614 ± 134 μmol TE/g) and coffee (*Coffea arabica*) beans (3511 ± 57 μmol TE/g) [76]. However our juice activity is close to the one observed for ginger rhizomes (*Zingiber officinalis;* 370 ± 28 μmol TE/g) [76], and higher than those reported for cherries (37.3–6.6 μmol TE/g) and blueberries (46.8–8.9 μmol TE/g) [77]. The antioxidant activity obtained for *P. friedrichsthalianum* juice supports previous results [6].

Regarding animal microbiota processing of plant components, hippurate has been considered the main excretion metabolic product of the biotransformation of phytochemicals in mammals [78] and its biosynthesis involves the hepatic condensation of glycine and benzoate [79]. Benzoate is one of the main microbial byproducts of a wide variety of chemical compounds from plant origin [80]. Increased hippurate excretion has been reported after phytochemical and plant extract ingestion in humans [81–84] and several animals [48,71,72]. However, no main changes in benzoate or glycine excretion have been reported in most of those intervention reports [48,71,81–84]. Cranberry administration in rats for 35 day-periods, has been associated with increased hippurate and benzoyl-glucuronide urinary excretion and with a reduction in several phytochemical and metabolic byproducts in the urine [72].

Considering that an increase in microbial degradation of phytochemicals has been related to augmented excretion of both, hippurate and benzoate [85], the observed higher urinary excretion of hippurate with a concomitant reduction in glycine and benzoate excretion may suggest an effect on the hepatic turnover of metabolic byproducts and not only an increase in phytochemical degradation. However, benzoylglucuronide in both rats and humans has been suggested to be a secondary byproduct at high doses of benzoate (> 500 mg/kg) over the saturation threshold of glycine conjugation (> 120 mg benzoate/kg) [86]. In general, hippurate excretion has been associated mainly with microbial presence [87,88].

Whereas no significant differences in hippurate excretion during the acute intervention were observed, there were changes after *P. friedrichsthalianum* juice short-term administration. This effect may not be only related to the metabolism of naturally occurring compounds from the juice, but probably also to the degradation of other phytochemicals presented in the food or even as the result of other normal liver detoxification processes. For instance, effects have been reported in rats on the bioavailability of naturally-occurring plant compounds, such as those present in commercial diets for laboratory animals [89]. Also, an eventual effect of short-term administration on gut microbiota could be related to changes in hippurate excretion. Changes in gut microbiota have been previously reported after phytochemical-rich feeding trials in rodents [90].

The same commercial diet was provided to animals during both, acute and short-term administration of *P. friedrichsthalianum.* However, a time-dependent exposure to *P. friedrichsthalianum* could have a differential effect, either in the regulation of xenobiotic handling or microbial transformation of phytochemicals present in the food or the juice itself as reported previously [89].

Chemical composition of *P. friedrichsthalianum* has shown the presence of quercetin, ellagitannins, gallic acid derivatives and quercetin glucosides, among other phenolic compounds [7], therefore collection periods for acute intervention were defined according to previous experiences and similar studies. Quercetin administration has been previously reported to be related to changes in urinary metabolome between 0 and 12 h, with a recovery period between 12 and 24 h [48]. Tea catechins, on the other hand, are mainly excreted in human urine after 6 h of administration [91].

Cranberry juice administration in rats has been associated with increased urinary excretion of phytochemicals and metabolic byproducts in the period from 0 to 8 h after the intervention [72] while administration of resveratrol in rats is related with main changes in endogenous metabolome in the first 12 h after administration [49]. Based on that, collection periods longer than 24 h were not considered in this study. For short-term intervention, collection periods were defined according to previous experiences from our group, using other phytochemicals and/or plant extracts [89].

In summary, our results suggest that *P. friedrichsthalianum* combination of phytochemicals could affect oxidative metabolism due to a modulation of redox balance in rats. Fig. 2 shows the interplay between ROS and the Krebs cycle, the regulation of the oxidative metabolism and our data from the short-term administration of *P. friedrichsthalianum* juice. Whether the observed effect is associated with intact phytochemicals present in the juice or modulated by secondary metabolites derived from the metabolism of the rats or their microbiota should be addressed in future investigations to complement the data presented here.

4. Conclusions

Administration of *P. friedrichsthalianum* fruit pulp juice in Wistar rats induced changes in intermediates of energy metabolism in acute and short-term intervention experiments, suggesting a potential role of some phytochemicals in the regulation of the *in vivo* redox balance. The acute intervention was mainly related to a reduction in the excretion of energy intermediaries, however, metabolites with significant reduction varied in different collection periods. Citrate and allantoin showed reduced excretion in the 0 to 6 h period while citrate and α-ketoglutarate were reduced in the 6 to 12 h period. Also, lactate and succinate showed reduced excretion in the 12 to 24 h period. Excretion of acetate increased in the 0 to 6 h but decreased in the 12 to 24 h period.

The short-term intervention was also associated with a decreased excretion of intermediates citrate, lactate, allantoin, succinate and α-ketoglutarate. The existence of a stronger effect after short-term administration suggests a time/dose-dependent relation and metabolic responses to the juice components. In summary, this study presents *in vivo* evidence that suggests a potential role for *P. friedrichsthalianum* juice phytochemicals in metabolic and redox balance, as previously suggested by *in vitro* studies.

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Author contribution

All authors contributed to the study conception and design. Material preparation and data collection were performed by Alexander Montoya-Arroyo, Giselle Tamayo-Castillo. Data Analysis was performed by Alexander Montoya-Arroyo, Giselle Tamayo-Castillo and Cecilia Díaz. The first draft of the manuscript was written by Alexander Montoya-Arroyo and all authors commented on the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare to have no conflict of interest regarding this investigation.

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Appendix A. Supplementary data

Supplementary data includes Two-dimensional NMR spectra to support the assignation of identified urinary metabolites. Two-dimensional spectra include HMBC, HSQC and dqCOSY. **Supplementary data to this article can be found online at** https://doi.org/10.1016/j.nfs.2020.07.003**.**

References

[1] A. Hjartåker, M.D. Knudsen, S. Tretli, E. Weiderpass, Consumption of berries, fruits and vegetables and mortality among 10,000 Norwegian men followed for four decades, Eur. J. Nutr. 54 (2015) 599–608, https://doi.org/10.1007/s00394-014-0741-9.

[2] X. Wang, Y. Ouyang, J. Liu, M. Zhu, G. Zhao, W. Bao, F.B. Hu, Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies, Bmj. 349 (2014) g4490, https://doi.org/10.1136/bmj.g4490.

[3] M.E. Díaz-Rubio, J. Pérez-Jiménez, M.Á. Martínez-Bartolomé, I. Álvarez, F. Saura-Calixto, Regular consumption of an antioxidant-rich juice improves oxidative status and causes metabolome changes in healthy adults, Plant Foods Hum. Nutr. 70 (2015) 9–14, https://doi.org/10.1007/s11130-014-0455-4.

[4] F. Brighenti, S. Valtueña, N. Pellegrini, D. Ardigò, D. Del Rio, S. Salvatore, P. Piatti, M. Serafini, I. Zavaroni, Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity C-reactive protein in adult Italian subjects, Br. J. Nutr. 93 (2005) 619, https://doi.org/10.1079/BJN20051400.

[5] J.A. Pino, R. Marbot, C. Vázquez, Characterization of Volatiles in Costa Rican Guava [*Psidium friedrichsthalianum* (Berg) Niedenzu] Fruit, J. Agric. Food Chem. 50 (2002) 6023–6026, https://doi.org/10.1021/jf011456i.

[6] G. Flores, K. Dastmalchi, S.-B. Wu, K. Whalen, A.J. Dabo, K.A. Reynertson, R.F. Foronjy, J.M.D. Armiento, E.J. Kennelly, Phenolic-rich extract from the Costa Rican guava (*Psidium friedrichsthalianum*) pulp with antioxidant and anti-inflammatory activity. Potential for COPD therapy, Food Chem. 141 (2013) 889–895, https://doi.org/10.1016/j.foodchem.2013.03.025.

[7] C. Rojas-Garbanzo, J. Winter, M.L. Montero, B.F. Zimmermann, A. Schieber, Characterization of phytochemicals in Costa Rican guava (*Psidium friedrichsthalianum* -Nied.) fruit and stability of main compounds during juice processing - (U)HPLC-DAD-ESI-TQD-MSn, J. Food Compos. Anal. 75 (2019) 26–42, https://doi.org/10.1016/j.jfca.2018.09.012.

[8] G. Flores, S.-B. Wu, A. Negrin, E.J. Kennelly, Chemical composition and antioxidant activity of seven cultivars of guava (Psidium guajava) fruits, Food Chem. 170 (2015) 327–335, https://doi.org/10.1016/j.foodchem.2014.08.076.

[9] C. Rojas-Garbanzo, B.F. Zimmermann, N. Schulze-Kaysers, A. Schieber, Characterization of phenolic and other polar compounds in peel and flesh of pink guava (*Psidium guajava* L. cv. 'Criolla') by ultra-high performance liquid chromatography with diode array and mass spectrometric detection, Food Res. Int. 100 (2017) 445–453, https://doi.org/10.1016/j.foodres.2016.12.004.

[10] K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, D. Hawkins Byrne, Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts, J. Food Compos. Anal. 19 (2006) 669–675, https://doi.org/10.1016/j.jfca.2006.01.003.

[11] F.M.V. Farinazzi-machado, É.L. Guiguer, S.M. Barbalho, S. De Souza, P. Cincotto, C. Gregório, A.C. Araújo, A. Rezende, T. Rodrigues, L.M. De, L. Lima, N.S. Marim, A. Cláudia, S. Brunnati, P. Martins, I.T. Sato, Effects of *Psidium guajava* on the metabolic profile of Wister rats, J. Med. Plant Res. 6 (2012) 3450–3454, https://doi.org/10.5897/JMPR12.033.

[12] V.M. Freitas, V.R. Correa, F.C. Motta, M.G. Sousa, A.C.M.M. Gomes, M.D.G. Carneiro, D.B. Silva, J.K. Mattos, M. Nicole, R.M.D.G. Carneiro, Resistant accessions of wild Psidium spp. to Meloidogyne enterolobii and histological characterization of resistance, Plant Pathol. 63 (2014) 738–746, https://doi.org/10.1111/ppa.12149.

[13] M. Marín, A. Casassa, A. Rincón, J. Labarca, Y. Hernández, Comportamiento de tipos de guayabo (*Psidium guajava* L .) , injertados sobre Psidium Performance of kinds guava (*Psidium guajava* L) grafted on *Psidium friedrichsthalianum* Berg-Niedenzu, (2000) 384–392.

[14] D.P. Jones, Y. Park, T.R. Ziegler, Nutritional metabolomics: progress in addressing complexity in diet and health, Annu. Rev. Nutr. 32 (2012) 183–202, https://doi.org/10.1146/annurev-nutr-072610-145159.

[15] A. Lafaye, C. Junot, B. Ramounet-Le Gall, P. Fritsch, J.-C. Tabet, E. Ezan, Metabolite profiling in rat urine by liquid chromatography/electrospray ion trap mass spectrometry. Application to the study of heavy metal toxicity, Rapid Commun. Mass Spectrom. 17 (2003) 2541–2549, https://doi.org/10.1002/rcm.1243.

[16] A.H. Emwas, R. Roy, R.T. McKay, D. Ryan, L. Brennan, L. Tenori, C. Luchinat, X. Gao, A.C. Zeri, G.A.N. Gowda, D. Raftery, C. Steinbeck, R.M. Salek, D.S. Wishart, Recommendations and standardization of biomarker quantification using NMR-based metabolomics with particular focus on urinary analysis, J. Proteome Res. 15 (2016) 360–373, https://doi.org/10.1021/acs.jproteome.5b00885.

[17] A. Zhang, H. Sun, X. Wu, X. Wang, Urine metabolomics, Clin. Chim. Acta 414 (2012) 65–69, https://doi.org/10.1016/j.cca.2012.08.016.

[18] F. Savorani, M.A. Rasmussen, M.S. Mikkelsen, S.B. Engelsen, A primer to nutritional metabolomics by NMR spectroscopy and chemometrics, Food Res. Int. 54 (2013) 1131–1145, https://doi.org/10.1016/j.foodres.2012.12.025.

[19] H.R. Eghbalnia, P.R. Romero, W.M. Westler, K. Baskaran, E.L. Ulrich, J.L. Markley, Increasing rigor in NMR-based metabolomics through validated and open source tools, Curr. Opin. Biotechnol. 43 (2017) 56–61, https://doi.org/10.1016/j.copbio.2016.08.005.

[20] R. Verpoorte, Y.H. Choi, N.R. Mustafa, H.K. Kim, Metabolomics: Back to basics, Phytochem. Rev. 7 (2008) 525–537, https://doi.org/10.1007/s11101-008-9091-7.

[21] D. Harman, Free radical theory of aging: an update - increasing the functional life span, Ann. N. Y. Acad. Sci. 1067 (2006) 10–21, https://doi.org/10.1196/annals.1354.003.

[22] D.P. Jones, Redox theory of aging, Redox Biol. 5 (2015) 71–79, https://doi.org/10.1016/j.redox.2015.03.004.

[23] A.L. Bulteau, M. Ikeda-Saito, L.I. Szweda, Redox-dependent modulation of aconitase activity in intact mitochondria, Biochemistry. 42 (2003) 14846–14855, https://doi.org/10.1021/bi0353979.

[24] L. Tretter, V. Adam-Vizi, Inhibition of Krebs cycle enzymes by hydrogen peroxide: a key role of α-ketoglutarate dehydrogenase in limiting NADH production under oxidative stress, J. Neurosci. 20 (2000) 8972–8979, https://doi.org/10.1523/JNEUROSCI.20-24-08972.2000.

[25] H.J. Forman, K.J.A. Davies, F. Ursini, How do nutritional antioxidants really work: nucleophilic tone and para-hormesis versus free radical scavenging in vivo, Free Radic. Biol. Med. 66 (2014) 24–35, https://doi.org/10.1016/j.freeradbiomed.2013.05.045.

[26] M.S. Elgawish, N. Kishikawa, M.A. Helal, K. Ohyama, N. Kuroda, Molecular modeling and spectroscopic study of quinone–protein adducts: insight into toxicity, selectivity, and reversibility, Toxicol. Res. 4 (2015) 843–847, https://doi.org/10.1039/C5TX00098J.

[27] Y. Li, S. Jongberg, M.L. Andersen, M.J. Davies, M.N. Lund, Quinone-induced protein modifications: kinetic preference for reaction of 1,2-benzoquinones with thiol groups in proteins, Free Radic. Biol. Med. 97 (2016) 148–157, https://doi.org/10.1016/j.freeradbiomed.2016.05.019.

[28] B. Groitl, U. Jakob, Thiol-based redox switches, Biochim. Biophys. Acta, Proteins Proteomics 1844 (2014) 1335–1343, https://doi.org/10.1016/j.bbapap.2014.03.007.

[29] M. Llana-Ruiz-Cabello, D. Gutiérrez-Praena, M. Puerto, S. Pichardo, Á. Jos, A.M. Cameán, In vitro pro-oxidant/antioxidant role of carvacrol, thymol and their mixture in the intestinal Caco-2 cell line, Toxicol. in Vitro 29 (2015) 647–656, https://doi.org/10.1016/j.tiv.2015.02.006.

[30] S. Camandola, M.P. Mattson, Brain metabolism in health, aging, and neurodegeneration, EMBO J. 36 (2017) 1474–1492, https://doi.org/10.15252/embj.201695810.

[31] R. Ventura-Clapier, A. Garnier, V. Veksler, F. Joubert, Bioenergetics of the failing heart, Biochim. Biophys. Acta, Mol. Cell Res. 1813 (2011) 1360–1372, https://doi.org/10.1016/j.bbamcr.2010.09.006.

[32] M. Hasanpour, M. Iranshahy, M. Iranshahi, The application of metabolomics in investigating anti-diabetic activity of medicinal plants, Biomed. Pharmacother. 128 (2020) 110263, https://doi.org/10.1016/j.biopha.2020.110263.

[33] N.N. Pavlova, C.B. Thompson, The emerging hallmarks of cancer metabolism, Cell Metab. 23 (2016) 27–47, https://doi.org/10.1016/j.cmet.2015.12.006.

[34] O. Beckonert, H.C. Keun, T.M.D. Ebbels, J. Bundy, E. Holmes, J.C. Lindon, J.K. Nicholson, Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts, Nat. Protoc. 2 (2007) 2692–2703, https://doi.org/10.1038/nprot.2007.376.

[35] E.J. Saude, B.D. Sykes, Urine stability for metabolomic studies: effects of preparation and storage, Metabolomics. 3 (2007) 19–27, https://doi.org/10.1007/s11306-006-0042-2.

[36] J.S. McKenzie, J.A. Donarski, J.C. Wilson, A.J. Charlton, Analysis of complex mixtures using high-resolution nuclear magnetic resonance spectroscopy and chemometrics, Prog. Nucl. Magn. Reson. Spectrosc. 59 (2011) 336–359, https://doi.org/10.1016/j.pnmrs.2011.04.003.

[37] H.C. Keun, T.M.D. Ebbels, H. Antti, M.E. Bollard, O. Beckonert, G. Schlotterbeck, H. Senn, U. Niederhauser, E. Holmes, J.C. Lindon, J.K. Nicholson, Analytical reproducibility in1H NMR-based metabonomic urinalysis, Chem. Res. Toxicol. 15 (2002) 1380–1386, https://doi.org/10.1021/tx0255774.

[38] T.M.D. Ebbels, E. Holmes, J.C. Lindon, J.K. Nicholson, Evaluation of metabolic variation in normal rat strains from a statistical analysis of 1H NMR spectra of urine, J. Pharm. Biomed. Anal. 36 (2004) 823–833, https://doi.org/10.1016/j.jpba.2004.08.016.

[39] R.E. Williams, E.M. Lenz, J.A. Evans, I.D. Wilson, J.H. Granger, R.S. Plumb, C.L. Stumpf, A combined (1)H NMR and HPLC-MS-based metabonomic study of urine from obese (fa/fa) Zucker and normal Wistar-derived rats, J. Pharm. Biomed. Anal. 38 (2005) 465–471, https://doi.org/10.1016/j.jpba.2005.01.013.

[40] Y. Wu, L. Li, Sample normalization methods in quantitative metabolomics, J. Chromatogr. A 1430 (2016) 80–95, https://doi.org/10.1016/j.chroma.2015.12.007.

[41] D.S. Wishart, T. Jewison, A.C. Guo, M. Wilson, C. Knox, Y. Liu, Y. Djoumbou, R. Mandal, F. Aziat, E. Dong, S. Bouatra, I. Sinelnikov, D. Arndt, J. Xia, P. Liu, F. Yallou, T. Bjorndahl, R. Perez-Pineiro, R. Eisner, F. Allen, V. Neveu, R. Greiner, A. Scalbert, HMDB 3.0-the human metabolome database in 2013, Nucleic Acids Res. 41 (2013) 801–807, https://doi.org/10.1093/nar/gks1065.

[42] C.C. Chyau, S.Y. Chen, C.M. Wu, Differences of Volatile and Nonvolatile Constituents between Mature and Ripe Guava (*Psidium guajava* Linn.) Fruits, J. Agric. Food Chem. 40 (1992) 846–849, https://doi.org/10.1021/jf00017a028.

[43] D. Morton, P. Griffiths, Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment, Vet. Rec. 116 (1985) 431–436, https://doi.org/10.1136/vr.116.16.431.

[44] J.M. Fentener van Vlissingen, M. Borrens, A. Girod, P. Lelovas, F. Morrison, Y.S. Torres, The reporting of clinical signs in laboratory animals: FELASA working group report, Lab. Anim. 49 (2015) 267–283, https://doi.org/10.1177/0023677215584249.

[45] L.L. Hamm, Renal handling of citrate, Kidney Int. 38 (1990) 728–735, https://doi.org/10.1038/ki.1990.265.

[46] L.K. Schnackenberg, J. Sun, P. Espandiari, R.D. Holland, J. Hanig, R.D. Beger, Metabonomics evaluations of age-related changes in the urinary compositions of male Sprague Dawley rats and effects of data normalization methods on statistical and quantitative analysis, BMC Bioinformatics. 8 (2007) 1–14, https://doi.org/10.1186/1471-2105-8-S7-S3.
[47] R. Beltrán-Debón, E. Rodríguez-Gallego, S. Fernández-Arroyo, O. Senan-Campos, F.A. Massucci, A. Hernández-Aguilera, M. Sales-Pardo, R. Guimerà, J. Camps, J.A. Menendez, J. Joven, The acute impact of polyphenols from Hibiscus sabdariffa in metabolic homeostasis: An approach combining metabolomics and gene-expression analyses, Food Funct. 6 (2015) 2957–2966, https://doi.org/10.1039/c5fo00696a.
[48] D. An, Q. Zhang, S. Wu, J. Wei, J. Yang, F. Dong, X. Yan, C. Guo, Changes of metabolic profiles in urine after oral administration of quercetin in rats, Food Chem. Toxicol. 48 (2010) 1521–1527, https://doi.org/10.1016/j.fct.2010.03.019.
[49] G. Torres Santiago, J.I. Serrano Contreras, M.E. Meléndez Camargo, L.G. Zepeda Vallejo, NMR-based metabonomic approach reveals changes in the urinary and fecal metabolome caused by resveratrol, J. Pharm. Biomed. Anal. 162 (2019) 234–241, https://doi.org/10.1016/j.jpba.2018.09.025.
[50] L.E. Dansie, S. Reeves, K. Miller, S.P. Zano, M. Frank, C. Pate, J. Wang, S. Jackowski, Physiological roles of the pantothenate kinases, Biochem. Soc. Trans. 42 (2014) 1033–1036, https://doi.org/10.1042/bst20140096.
[51] S.J. Hayflick, Defective pantothenate metabolism and neurodegeneration, Biochem. Soc. Trans. 42 (2014) 1063–1068, https://doi.org/10.1042/BST20140098.
[52] O. Halvorsen, S. Skrede, Regulation of the biosynthesis of CoA at the level of pantothenate kinase, Eur. J. Biochem. 124 (1982) 211–215 http://www.ncbi.nlm.nih.gov/pubmed/7084227.
[53] V. Leoni, L. Strittmatter, G. Zorzi, F. Zibordi, S. Dusi, B. Garavaglia, P. Venco, C. Caccia, A.L. Souza, A. Deik, C.B. Clish, M. Rimoldi, E. Ciusani, E. Bertini, N. Nardocci, V.K. Mootha, V. Tiranti, Metabolic consequences of mitochondrial coenzyme A deficiency in patients with PANK2 mutations, Mol. Genet. Metab. 105 (2012) 463–471, https://doi.org/10.1016/j.ymgme.2011.12.005.
[54] R. Leonardi, Y.M. Zhang, M.K. Yun, R. Zhou, F.Y. Zeng, W. Lin, J. Cui, T. Chen, C.O. Rock, S.W. White, S. Jackowski, Modulation of pantothenate kinase 3 activity by small molecules that interact with the substrate/allosteric regulatory domain, Chem. Biol. 17 (2010) 892–902, https://doi.org/10.1016/j.chembiol.2010.06.006.
[55] R. Leonardi, C.O. Rock, S. Jackowski, Y.-M. Zhang, Activation of human mitochondrial pantothenate kinase 2 by palmitoylcarnitine, Proc. Natl. Acad. Sci. 104 (2007) 1494–1499, https://doi.org/10.1073/pnas.0607621104.
[56] C.O. Rock, R.B. Calder, M.A. Karim, S. Jackowski, Pantothenate kinase regulation of the intracellular concentration of coenzyme a, J. Biol. Chem. 275 (2000) 1377–1383, https://doi.org/10.1074/jbc.275.2.1377.
[57] S. Dubuis, K. Ortmayr, M. Zampieri, A framework for large-scale metabolome drug profiling links coenzyme A metabolism to the toxicity of anti-cancer drug dichloroacetate, Commun. Biol. 1 (2018) 1–11, https://doi.org/10.1038/s42003-018-0111-x.
[58] B.S. Ferguson, M.J. Rogatzki, M.L. Goodwin, D.A. Kane, Z. Rightmire, L.B. Gladden, Lactate Metabolism: Historical Context, Prior Misinterpretations, and Current Understanding, Springer, Berlin Heidelberg, 2018, https://doi.org/10.1007/s00421-017-3795-6.
[59] T. Hashimoto, Colocalization of MCT1, CD147, and LDH in mitochondrial inner membrane of L6 muscle cells: evidence of a mitochondrial lactate oxidation complex, AJP Endocrinol. Metab. 290 (2006) E1237–E1244, https://doi.org/10.1152/ajpendo.00594.2005.
[60] W. Tiemeyer, D. Giesecke, Quantitative determination of allantoin in biological fluids by reversed-phase high-pressure liquid chromatography, Anal. Biochem. 123 (1982) 11–13, https://doi.org/10.1016/0003-2697(82)90616-9.
[61] B. Halliwell, M. Whiteman, Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br. J. Pharmacol. 142 (2004) 231–255, https://doi.org/10.1038/sj.bjp.0705776.
[62] M. Oda, Y. Satta, O. Takenaka, N. Takahata, Loss of urate oxidase activity in hominoids and its evolutionary implications, Mol. Biol. Evol. 19 (2002) 640–653, https://doi.org/10.1093/oxfordjournals.molbev.a004123.
[63] L.S. Raab, G.L. Decker, A.J. Jonas, M.A. Kaetzel, J.R. Dedman, Glucocorticoid regulation of rat liver urate oxidase, J. Cell. Biochem. 47 (1991) 18–30, https://doi.org/10.1002/jcb.240470104.
[64] R.G. Hunter, M. Seligsohn, T.G. Rubin, B.B. Griffiths, Y. Ozdemir, D.W. Pfaff, N.A. Datson, B.S. McEwen, Stress and corticosteroids regulate rat hippocampal mitochondrial DNA gene expression via the glucocorticoid receptor, Proc. Natl. Acad. Sci. 113 (2016) 9099–9104, https://doi.org/10.1073/pnas.1602185113.
[65] N. Pak, G. Donoso, M.A. Tagle, Allantoin excretion in the rat, Br. J. Nutr. 30 (1973) 107–112, https://doi.org/10.1079/BJN19730012.
[66] E. Pouteau, I. Meirim, S. Métairon, L.B. Fay, Acetate, propionate and butyrate in plasma: determination of the concentration and isotopic enrichment by gas chromatography/mass spectrometry with positive chemical ionization, J. Mass Spectrom. 36 (2001) 798–805, https://doi.org/10.1002/jms.181.
[67] S.E. Knowles, I.G. Jarrett, O.H. Filsell, F.J. Ballard, Production and utilization of acetate in mammals, Biochem. J. 142 (1974) 401–411, https://doi.org/10.1042/BJ1420401.
[68] A.O. Akanji, S. Humphreys, V. Thursfield, T.D. Hockaday, The relationship of plasma acetate with glucose and other blood intermediary metabolites in non-diabetic and diabetic subjects, Clin. Chim. Acta 185 (1989) 25–34, https://doi.org/10.1016/0009-8981(89)90127-7.
[69] D.K. Deelchand, A.A. Shestov, D.M. Koski, K. Uğurbil, P.G. Henry, Acetate transport and utilization in the rat brain, J. Neurochem. 109 (2009) 46–54, https://doi.org/10.1111/j.1471-4159.2009.05895.x.
[70] S. Dall'Acqua, M. Stocchero, M. Clauser, I. Boschiero, E. Ndoum, M. Schiavon, S. Mammi, E. Schievano, Changes in urinary metabolic profile after oral administration of curcuma extract in rats, J. Pharm. Biomed. Anal. 100 (2014) 348–356, https://doi.org/10.1016/j.jpba.2014.07.035.
[71] S. Dall'Acqua, M. Stocchero, I. Boschiero, M. Schiavon, S. Golob, J. Uddin, D. Voinovich, S. Mammi, E. Schievano, New findings on the in vivo antioxidant activity of Curcuma longa extract by an integrated 1H NMR and HPLC–MS metabolomic approach, Fitoterapia. 109 (2016) 125–131, https://doi.org/10.1016/j.fitote.2015.12.013.
[72] G. Peron, A. Pellizzaro, P. Brun, E. Schievano, S. Mammi, S. Sut, I. Castagliuolo, S. Dall'Acqua, Antiadhesive activity and metabolomics analysis of rat urine after cranberry (Vaccinium macrocarpon Aiton) administration, J. Agric. Food Chem. 65 (2017) 5657–5667, https://doi.org/10.1021/acs.jafc.7b01856.
[73] C.M. Bitencourt, C.S. Fávaro-Trindade, P.J.A. Sobral, R.A. Carvalho, Gelatin-based films additivated with curcuma ethanol extract: antioxidant activity and physical properties of films, Food Hydrocoll. 40 (2014) 145–152, https://doi.org/10.1016/j.foodhyd.2014.02.014.
[74] A. Floegel, D.-O. Kim, S.-J. Chung, S.I. Koo, O.K. Chun, Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods, J. Food Compos. Anal. 24 (2011) 1043–1048, https://doi.org/10.1016/j.jfca.2011.01.008.
[75] N.P. Seeram, S.M. Henning, Y. Niu, R. Lee, H.S. Scheuller, D. Heber, Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity, J. Agric. Food Chem. 54 (2006) 1599–1603, https://doi.org/10.1021/jf052857r.
[76] S. Dudonné, X. Vitrac, P. Coutière, M. Woillez, J.-M. Mérillon, Comparative study of antioxidant properties and Total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays, J. Agric. Food Chem. 57 (2009) 1768–1774, https://doi.org/10.1021/jf803011r.
[77] H. Speisky, C. López-Alarcón, M. Gómez, J. Fuentes, C. Sandoval-Acuña, First web-based database on total phenolics and oxygen radical absorbance capacity (ORAC) of fruits produced and consumed within the South Andes region of South America, J. Agric. Food Chem. 60 (2012) 8851–8859, https://doi.org/10.1021/jf205167k.
[78] H.J. Lees, J.R. Swann, I.D. Wilson, J.K. Nicholson, E. Holmes, Hippurate: the natural history of a mammalian-microbial cometabolite, J. Proteome Res. 12 (2013) 1527–1546, https://doi.org/10.1021/pr300900b.
[79] S.J. Gatley, H.S. Sherratt, The synthesis of hippurate from benzoate and glycine by rat liver mitochondria. Submitochondrial localization and kinetics, Biochem. J. 166 (1977) 39–47, https://doi.org/10.1042/bj1660039.
[80] B.L. Goodwin, C.R.J. Ruthven, M. Sandler, Gut flora and the origin of some urinary aromatic phenolic compounds, Biochem. Pharmacol. 47 (1994) 2294–2297, https://doi.org/10.1016/0006-2952(94)90268-2.
[81] F.A. Van Dorsten, C.A. Daykin, T.P.J. Mulder, J.P.M. Van Duynhoven, Metabonomics approach to determine metabolic differences between green tea and black tea consumption, J. Agric. Food Chem. 54 (2006) 6929–6938, https://doi.org/10.1021/jf061016x.
[82] A.R. Rechner, G. Kuhnle, P. Bremner, G.P. Hubbard, K.P. Moore, C.A. Rice-Evans, The metabolic fate of dietary polyphenols in humans, Free Radic. Biol. Med. 33 (2002) 220–235, https://doi.org/10.1016/S0891-5849(02)00877-8.
[83] M.C. Walsh, L. Brennan, E. Pujos-Guillot, J.L. Sébédio, A. Scalbert, A. Fagan, D.G. Higgins, M.J. Gibney, Influence of acute phytochemical intake on human urinary metabolomic profiles, Am. J. Clin. Nutr. 86 (2007) 1687–1693, https://doi.org/10.1093/ajcn/86.6.1687.
[84] C.A. Daykin, J.P.M. Van Duynhoven, A. Groenewegen, M. Dachtler, J.M.M. Van Amelsvoort, T.P.J. Mulder, Nuclear magnetic resonance spectroscopic based studies of the metabolism of black tea polyphenols in humans, J. Agric. Food Chem. 53 (2005) 1428–1434, https://doi.org/10.1021/jf048439o.
[85] R.S. Lord, J.A. Bralley, Clinical applications of urinary organic acids. part 1: Detoxification markers, Altern. Med. Rev. 13 (2008) 205–215.
[86] K. Zu, D.M. Pizzurro, T.A. Lewandowski, J.E. Goodman, Pharmacokinetic data reduce uncertainty in the acceptable daily intake for benzoic acid and its salts, Regul. Toxicol. Pharmacol. 89 (2017) 83–94, https://doi.org/10.1016/j.yrtph.2017.07.012.
[87] H.R.T. Williams, I.J. Cox, D.G. Walker, J.F.L. Cobbold, S.D. Taylor-Robinson, S.E. Marshall, T.R. Orchard, Differences in gut microbial metabolism are responsible for reduced hippurate synthesis in Crohn's disease, BMC Gastroenterol. 10 (2010) 1–8, https://doi.org/10.1186/1471-230X-10-108.
[88] T. Pallister, M.A. Jackson, T.C. Martin, J. Zierer, A. Jennings, R.P. Mohney, A. MacGregor, C.J. Steves, A. Cassidy, T.D. Spector, C. Menni, Hippurate as a metabolomic marker of gut microbiome diversity: modulation by diet and relationship to metabolic syndrome, Sci. Rep. 7 (2017) 1–9, https://doi.org/10.1038/s41598-017-13722-4.
[89] J.-M. Fallas-Ramirez, L. Hernandez, F. Vaillant, Untargeted metabolomic profiling of urine in Wistar rats reveals enhanced bioavailability of soy isoflavones post short-term consumption of noni (Morinda citrifolia) juice, J. Funct. Foods 40 (2018) 51–59, https://doi.org/10.1016/j.jff.2017.10.034.
[90] R.V. Espley, C.A. Butts, W.A. Laing, S. Martell, H. Smith, T.K. McGhie, J. Zhang, G. Paturi, D. Hedderley, A. Bovy, H.J. Schouten, J. Putterill, A.C. Allan, R.P. Hellens, Dietary flavonoids from modified apple reduce inflammation markers and modulate gut microbiota in mice, J. Nutr. 144 (2014) 146–154, https://doi.org/10.3945/jn.113.182659.
[91] C.S. Yang, L. Chen, M.J. Lee, D. Balentine, M.C. Kuo, S.P. Schantz, Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers, Cancer Epidemiol. Biomark. Prev. 7 (1998) 351–354 http://www.ncbi.nlm.nih.gov/pubmed/9568793.