Genipap (Genipa americana L.) juice intake biomarkers after medium-term consumption

Livia Dickson, Mathieu Tenon, Ljubica Svilar, Pascale Fanca-Berthon, Jean-Charles Martin, Herve Rogez, Fabrice Vaillant

Journal Pre-proofs

Genipap (*Genipa americana* L.) juice intake biomarkers after medium-term consumption

Livia Dickson, Mathieu Tenon, Ljubica Svilar, Pascale Fança-Berthon, Jean-Charles Martin, Hervé Rogez, Fabrice Vaillant

PII: S0963-9969(20)30400-2
DOI: https://doi.org/10.1016/j.foodres.2020.109375
Reference: FRIN 109375

To appear in: *Food Research International*

Received Date: 20 January 2020
Revised Date: 26 May 2020
Accepted Date: 2 June 2020

Please cite this article as: Dickson, L., Tenon, M., Svilar, L., Fança-Berthon, P., Martin, J-C., Rogez, H., Vaillant, F., Genipap (*Genipa americana* L.) juice intake biomarkers after medium-term consumption, *Food Research International* (2020), doi: https://doi.org/10.1016/j.foodres.2020.109375

Genipap (*Genipa americana* L.) juice intake biomarkers after medium-term consumption

Livia Dickson [a,b,c,d], Mathieu Tenon [b], Ljubica Svilar [e], Pascale Fança-Berthon [b], Jean-Charles Martin [e], Hervé Rogez [a] and Fabrice Vaillant [c,d*]

[a]Federal University of Pará & Centre for Valorization of Amazonian Bioactive Compounds (CVACBA), Parque de Ciência e Tecnologia Guamá, Av. Perimetral da Ciência, km 01, Guamá 66075-750, Brazil. email: livia_dickson@yahoo.com.br

[b]Naturex SA, 250 rue Pierre Bayle, BP81218, 84911 Avignon CEDEX 9, France. email: M. Tenon, m.tenon@naturex.com; P. Fança-Berthon, p.fancaberthon@naturex.com

[c]Centre International de Recherche Agronomique pour le Développement (CIRAD), Avenue Agropolis, TA50/PS4, 34398 Montpellier CEDEX 5, France. email: F. Vaillant, fabrice.vaillant@cirad.fr

[d]Qualisud, Univ Montpellier, CIRAD, Montpellier SupAgro, Univ d'Avignon, Univ de La Réunion, Montpellier, France.

[e]Aix Marseille Univ, INSERM, INRA, C2VN, CRIBIOM, 5-9, Boulevard Maurice Bourdet, CS 80501, 13205 Marseille CEDEX 01, France. email: J-C. Martin, jean-charles.martin@univ-amu.fr; L. Svilar, ljubica.svilar@univ-amu.fr

*Corresponding Author: Fabrice VAILLANT. phone: +33 91 3201-7456, email: fabrice.vaillant@cirad.fr

Abstract

Genipap (*Genipa americana* L.) is an exotic fruit largely consumed and well known, in Amazonian pharmacopeia, to treat anemia, measles and uterine cancer. It is also used as a diuretic, digestive, healing, laxative and antiseptic. The aim of this study was to apply an untargeted metabolomics strategy to determine biomarkers of food intake after short-term consumption of genipap juice. Sixteen healthy adult men were administered jenipap juice (250 mL) twice a day for three weeks. Before and after the three weeks of consumption. the subjects drank a control drink, and they

consumed a standard diet. Urine was collected after 0-6 h, 6-12 h and 12-24 h. An ultrahigh-performance liquid chromatography-mass spectrometry (UHPLC-MS)-based metabolomics approach was applied to analyze the urine samples. Principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed to highlight experimental differences between groups. The value of the area under the curve (AUC) of the receiver operator characteristic (ROC) curve validated the identified biomarkers. Thirty-one statistically affected urinary metabolites were putatively identified and were mainly related to iridoids family, medium-chain fatty acids, and polyphenols. Also a group of urinary markers including dihydrocaffeic acid (DHCA), 1-(4-hydroxyphenyl)-1,2-propanediol and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid were established as biomarkers of genipap consumption. Our findings have established a comprehensive panel of changes in the urinary metabolome and provided information to monitor endogenous alterations that are linked to genipap juice intake. These data should be used in further studies to understand the health implications of genipap juice consumption.

Keywords: untargeted metabolomics, urine, LC-MS, food biomarkers intake, iridoids, exotic fruit.

1. Introduction

Amazonia has a large number of unexplored native and exotic fruits with potential use in agroindustry and as a source of income for the local population (Rufino *et al*., 2010). Many of these fruits are largely consumed and are also recommended for use as folk medicine to treat and/or prevent disease. However, scientific data on bioactivity are still scarce for a large majority of these exotic fruits. Studies that can define a list of biomarkers of food intake (BFI) and prove their specific functionality for health are fundamental to add value to these products and provide new options of functional food products to the consumer (Rogez *et al*., 2004).

Genipap, or jagua (*Genipa americana* L.), is an exotic fruit native to the Amazon. The pulp contains 4.4 to 25.7% carbohydrates (de Souza *et al*., 2012; Morton, 1987), with reducing sugars predominating, on average, at 9.4%, nonreducing sugars at 4.5% and total sugars between 3.8 to 12.4% (Figueiredo *et al*., 1986; Hansen *et al*., 2008; Orwa *et al*., 2009). Fiber content varies from 1.9 to 9% (Silva *et al*., 2009; Pacheco *et al*., 2015). The protein content is approximately 0.5 to 5.2%, and that of lipids is 0 to 1.6%, which is largely composed of saturated (52.3%) and unsaturated fatty acids (25.6%) (Hamacek *et al*., 2013; Pacheco *et al*., 2015). With respect to minerals, the pulp (100 g sample) contains 1.9% potassium, 2.0% magnesium (de Souza *et al*., 2012), 8.5 to 17% thiamine, 18.5 to 37% riboflavin, and 3.4 to 5.2% niacin (J. F. Morton, [s.d.]; Villachica et al., 1996). Vitamin C content varies from 1.1 to 33 mg/100 g. Phenolic content can reach 857.1 mg GAE.100^{-1} g in dry samples (Porto *et al*., 2014), and iridoids in ripe fruit were observed at 0.2 ± 0.0 mg/g dry pulp (Bentes & Mercadante, 2014).

Some studies have suggested the bioactivity of this fruit. *In vitro* antioxidant (Hwa *et al*., 2011; Son *et al*., 2015), anticancer (Li *et al*., 2018; Shanmugam *et al*., 2018), immunomodulatory (Hwa *et al*., 2011; Son *et al*., 2015), placental cell regulation (Conceição *et al*., 2011) and neuroprotective (Liu *et al*., 2009) activities have already been shown. In animal models, other biological activities, such as choleretic (Mikami & Takikawa, 2008), anticancer (Li *et al*., 2018; Shanmugam *et al*., 2018), hepatoprotective (Wu *et al*., 2009), anti-inflammatory (Koo *et al*., 2004; Viljoen *et al*., 2012) and hypoglycemic (Wu *et al*., 2009) activities, have also been reported. Studies that characterized this fruit refer to it as a source of bioactive compounds, mainly iridoids, but bioavailability of these compounds and their potential impact on health are still unknown and controversial (Bentes & Mercadante, 2014; Conceição *et al*., 2011).

Described biological activities of the bioactive compounds present in this fruit are important to investigate in humans because biomarker of food intake allows us to understand better the interaction of food with the organism and eventually make diet–health associations. Thus, the metabolomic approach represents a good tool for this type of investigation. Metabolomics measures real parameters of physiological regulatory processes and gives more knowledge about the metabolic pathways, such as the regulatory process and the interaction with the environment. It is a signature of biochemical activity and is, so, more straightforward to correlate with the phenotype (Garcia-Aloy et al., 2014)

In a previous study, the metabolites of acute genipap ingestion were tentatively identified (Dickson *et al*., 2018). In this study, to investigate the effect of genipap juice intake on a medium-term timescale, we performed untargeted metabolomics analyses on urine from humans before and after three weeks of twice-daily genipap juice consumption. The objective of the present study was to search for reliable differences in the human urinary metabolome and in routine blood analysis results.

2. Materials and methods

2.1. Subjects

An open-label, crossover clinical trial was approved by the ethical committee of the Institute of Health Sciences of the Federal University of Para (Belém, Brazil; ethical ID: 1.436.134). The study was conducted in strict accordance with the international ethical guidelines for research involving humans established in the Declaration of Helsinki and in compliance with resolution 196/96 of the National Health Council of the Ministry of Health from Brazil. All participants provided written, informed consent.

2.2. Study design

The selection of the 16 subjects, precautions taken during the wash-out period and genipap juice preparation have been previously described (Dickson *et al*., 2018). The study was designed to include two days with urine sample collection (day 1 for baseline and day 23) separated by 3 weeks of daily genipap juice consumption totaling 500 ml, 250 ml after fasting in the morning, and 250 ml at the end of the afternoon (between 18:00 h and 19:00 h). For the baseline (day 1) and after the 3 weeks (day 23) drinking the juice volunteers drank 500 mL of a placebo (water with white

crystallized cane sugar (95:5; *w*: *w*)) after at least 12 h of fasting. Volunteers were asked to drink the placebo in a maximum lapse time of 20 min. During both days, the same standardized meals were provided to volunteers on a normal schedule. All urine samples were collected in 3 different bottles corresponding to 0–6 h, 6–12 h and 12–24 h after drinking. At each time point, 6 h, 12 h and 24 h, volunteers were asked to empty their bladder into the corresponding bottle before changing to a new one. Bottles were always kept under refrigeration, and immediately after completing the urine collection for each timeperiod, total volume was measured, aliquots taken from the bottles were stored at -80°C, and the rest of the urine was discarded.

2.3. Sample preparation and UHPLC-HRMS analysis

Urine samples were prepared as previously described (Dickson *et al*., 2018), thawed on ice, vortex mixed for 1 min and centrifuged at 11000×g for 15 min at 4 °C. An aliquot of 150 µL of supernatant was diluted with 1.200 µL Milli-Q water and vortex mixed for 30 sec. Samples were centrifuged again at 11000×g for 15 min at 4 °C, and the supernatant was kept at -80 °C until the day of analysis. An aliquot of 5 µL of each sample was injected into a UHPLC (Thermo Fisher Scientific, Courtaboeuf, France) and separated using a Hypersil Gold C18 column (100 mm × 2.1 mm, 1.9 µm particle size) with the temperature set at 40 °C. The column was eluted using a 16.0 min gradient with acetonitrile containing 0.1% formic acid (A) and water containing 0.1% formic acid (B) at a flow rate of 0.4 mL/min. Agradient program was as follows: 1 min, 100% B; 4.7 min, 80% B; 9.5 min, 25% B; 11 min, 0% B; 12 min, 0% B; and held up to 16 min. All samples were kept at 4°C during the analysis. MS analysis was performed using a Q Exactive Focus mass spectrometer (Thermo Fisher Scientific, Courtaboeuf, France) equipped with a heated electrospray ionization source (HESI). Spray voltage was set at 3500 V, and the HESI probe and transfer capillary temperature were kept at 310 °C and 320 °C, respectively. Sheath and auxiliary gas were maintained at 30 and 8 (arbitrary units), respectively, and the S-lens RF was set at 55 V. Analysis was performed in full MS scan mode with switching ionization polarity mode. resolution was set to 35000 full width at half maximum (FWHM) at *m/z* 200. Automatic gain control (AGC) was set at 1e6 with a maximum injection time (IT) of 250 ms. Full MS spectra were acquired in profile mode with the *m/z* range from 80 to 1000 *m/z*, and a collision energy of 40 eV. MS/MS experiments for metabolite identification of selected ions were performed, and collision energies of 10, 20 and 30 eV were applied.

2.4. Data preprocessing and statistical analysis

Preprocessing and statistical analysis used in this study has been described elsewhere (Dickson *et al*., 2018). Full MS raw data of both ionization polarities were extracted and preprocessed using XCMS (Colin *et al*., 2006) software in the R environment (R Core Team, 2013). Filtration and peak picking were performed using the xcmsSet function. CentWave function was used for peak detection. Times of 2 and 15 s were used as the minimal and maximal chromatographic peak widths, respectively. The minimum acceptable signal/noise was fixed at 3. A difference of 0.00005 *m/z* was used to consider peaks as having the same *m/z*. The maximal *m/z* deviation in consecutive scans was set at 5 ppm. The noise was standardized to a relative intensity of 1000, and the integration of the *m/z* centroid peak was performed through descent on Mexican hat filtered data. For alignment, the Obiwarp method was used, and the peaks were grouped using the density method. A bandwidth of 5 seconds and an *m/z* overlap width of 0.015 seconds were used to create peak density chromatograms. Ions that did not appear in a minimum fraction of 0.75 of the sample's groups were considered a nonvalid group. Additionally, ions that did not appear in at least 3 of the sample groups were excluded. The missing values were gap-filled with the fillPeaks method. To perform normalization to correct the drift between different series, quality controls consisting of pooled urine samples were used. Datasets obtained for positive and negative ionization modes were pretreated using Pareto scaling and log transformation. Univariate and multivariate statistical approaches were performed using Metaboanalyst (Xia & S Wishart, 2011) to explore the differences between sample groups. A parametric *t*-test was calculated by applying an adjusted value for the false discovery rate (FDR). All ions with a p-value < 0.05 (univariate) and a VIP > 1 (variable importance in projection (VIP) of partial least-square multivariate discriminant analysis) were considered statistically significant. In parallel, principal component analysis (PCA) was performed with all detected ions to explore group differences. Partial least-square discriminant analysis (PLS-DA) using SIMCA P (Sartorius Stedim Data Analytics AB, Umea, Sweden) was applied to assess the variation between groups. The predictive ability of the model was validated by cross validation ANOVA (CV-ANOVA), permutation tests (n=100) and receiver operating characteristic (ROC) curves. ROC generation was performed using the MetaboAnalyst web-based tool. Both individual and multiple biomarker ROC curves were built. The accuracy and sensitivity of ROC analysis were assessed using the area under the curve (AUC).

The interpretation of the AUC results was as follows: 0.9–1.0 = excellent; 0.8–0.9 = good; 0.7–0.8 = fair; 0.6–0.7 = poor; 0.5–0.6 = fail.

2.5. Metabolite identification and biological interpretation

To take into account all possible adducts and fragments, discriminant ions were clustered according to their retention time and similar intensity features across all the samples. The 50 most discriminant features in each ionization polarity were selected. Then, the identified parent ions were fragmented with collision energies of 10, 20, and 30 eV. When available, all datasets were matched with the *in-house* tool of the Workflow for Metabolomics web service (Caron *et al*., 2014). For other cases, the fragmentation pattern was compared to the experimental pattern reported in metabolite databases, including HMDB (Wishart *et al*., 2013) and METLIN (Smith *et al*., 2005), or to the in silico fragmentation pattern generated by online software, such as CFM-ID (Allen *et al*., 2014), MetFrag (Ruttkies *et al*., 2016) and MetFusion (Gerlich & Neumann, 2013). When good matches were obtained, a tentative annotation was reported as well as the confidence level of the identification suggested by the Metabolites Standards Initiative (Sumner *et al*., 2007).

Biological interpretation was accomplished through available information in the literature as well as with web-based software tools, such as BioCyc (Caspi *et al*., 2016), KEGG (Kanehisa & Goto, 2000), ChEBI (Degtyarenko *et al*., 2008), HMDB (Wishart *et al*., 2013), KNApSAcK (http://kanaya.naist.jp/KNApSAcK/) and Metabolite Set Enrichment Analysis (MSEA) (Persicke *et al*., 2012).

3. Results and discussion

After data LC-MS postprocessing, the urine analysis of sixteen volunteers after three weeks of genipap juice consumption showed 1051 and 975 features in positive and negative mode, respectively. Based on the *t*-test comparing the mean signal intensities before and after diet intervention, 142 and 121 features were significantly different in positive and negative ionization mode, respectively (P-value<0.05). 50 most discriminant features from each polarity were selected (Supplementary material, Tables S1 and S2). Among them, thirty ions were putatively identified in both ionization modes, with genipic acid detected in both positive and negative ionization modes. Comparison with the most discriminant metabolites identified in acute exposure to genipap

(Dickson *et al*., 2018) revealed that twenty-two metabolites were common in both interventions, which shows an important similarity between the pattern of metabolites excreted. **Figure 1** shows 30 urinary metabolites tentatively identified after medium-term consumption of genipap juice. Twenty-two (22) urinary metabolites were observed after both acute and medium-term consumption, and eight (8) were exclusive to the medium-term study. Nine (9) urinary biomarkers were exclusively detected in the acute study (Dickson *et al*., 2018). Biomarkers only detected after medium-term exposure to genipap juice were tentatively identified as L,L-cyclo(leucylprolyl), methylxanthine, pimelic acid, (Z)-3-(1-formyl-1-propenyl)pentanedioic acid, 3-(1-hydroxymethyl-1-propenyl)pentanedioic acid, vanillactic acid (VLA), 5-acetylamino-6-formylamino-3-methyluracil (AFMU), and 3-hexenoic acid (**Figure 1**).

Unsupervised PCA analysis comprising all detected features in both ionization modes clearly allowed for distinguishing the groups before and after medium-term genipap juice consumption (Supplementary material, Figure S2). Supervised PLS-DA analysis resulted in a robust model that was able to class subjects into the correct groups (**Figure 2A).** Model was validated by the cross-validation ANOVA *p*-value ($2.36\ e^{28}$), R^2Y (>0.8), Q^2Y (>0.75) and the permutation tests that showed no overfitting (**Figure 2B**).

Except for 8-hydroxygenipic acid, all compounds from the iridoid family derived from genipin and genipic acid were identified in both acute and medium-term exposure to genipap juice. Nonetheless, iridoid derivatives such as 11-deoxygenipic acid, genipin isomer, 3(4)-dehydrogenipic acid, hydroxymethyl-cyclopenta[c]furan-1,3diol, 14-deoxygenipinic acid, and 3(7)-dehydrogenipinic acid were much more intense after acute consumption (Dickson *et al*., 2018) than in this medium-term study. These results suggested that these compounds present in genipap juice were probably absorbed in the upper digestive tract and excreted relatively quickly. On the other hand, other metabolites, such as genipic acid and 12-demethylated-8-hydroxygenipinic acid, increased considerably after medium-term intervention (by 1000-fold, on average, for both compounds between acute and medium-term intervention). This fact suggests that microbial enzymes from the microbiota probably mediate the formation of these metabolites. Excretion of both iridoids showed high interindividual variability (**Figure 3**). Some individuals (4 out of 16 volunteers, individuals 19, 1, 28 and 7) showed very low excretion of genipic acid and 12-demethylated-8-hydroxygenipinic acid (D-8H genipinic acid). On the other hand, 1 volunteer

(individual 37) was able to excrete an average amount of genipic acid, but D-8H was almost undetectable, while another individual (number 16) showed the opposite, with relatively high excretion of D-8H genipinic acid but very low excretion of genipic acid. Individual 22 showed very high excretion of both metabolites, while most individuals (8 out 16) showed similar excretion patterns. Although the population studied was too small to draw a conclusion, these data suggest the presence of different metabotypes of microbiota with different abilities to mediate the formation of these iridoid metabolites.

After iridoids, the main urinary metabolites observed in acute and medium-term intervention were (1R,6R)-6-hydroxy-2-succinylcyclohexa-2,4-diene-1-carboxylate, cinnamic acid derivatives and CMPF (3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid). The tentatively identified metabolite (1R,6R)-6-hydroxy-2-succinylcyclohexa-2,4-diene-1-carboxylate is an intermediate of the vitamin K biosynthetic pathway. Dihydrocaffeic acid (DHCA) is a relatively common biomarker of polyphenol intake and has been reported as a biomarker of coffee, wine, fruits and vegetables (Huang, de Paulis, & May, 2004; Wittemer *et al*., 2005). Even though this compound is probably mainly absorbed in the upper gut tract, the excretion continued even 36 h after consumption of the juice, indicating absorption in the lower gut tract and probable mediation of microbiota. This result is in accordance with those of Wittemer *et al*. (2005), who detected DHCA in human urine long after oral administration of artichoke leaf extract.

3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) is one of the main metabolites of furan fatty acids that is reported as a robust biomarker of fish intake. Furan fatty acids (F-acids) are heterocyclic fatty acids characterized by a central furan ring that has an unbranched fatty acid chain (usually at positions 7, 9, 11, or 13) and an alkyl group. Widespread in food, F-acids are found in vegetables, fruits, seed oil, milk fat, butter, fish, and fish oil. In plants, F-acids are secondary metabolites produced in the process of defense against oxidative stress (Hanhineva et al., 2015; Sinclair et al., 2018). Mammals cannot synthesize these compounds, so they are introduced into the human body through food and the excretion of their metabolites can be observed in urine and blood. These compounds are considered valuable antioxidants, seem to inhibit the progression of non-enzymatic lipid peroxidation, and act in the elimination of radicals, which provides a protective effect against cardiovascular diseases and therapy for the treatment of other inflammatory disorders. Compounds present in the genipap may stimulate the degradation

pathway of furan fatty acids and, therefore, explain the CMPF increases. Excretion of CMPF in our samples increased after the medium-term consumption of genipap juice (Supplementary material Figure S3). Therefore, if in the control samples the levels of these compounds were low, this means that their increase is related to the genipap juice consumption. F-acids from food are incorporated into phospholipids and cholesterol esters and excreted in the urine. It is not yet known where F-acids are metabolized to CMPF (Xu et al., 2017). In mammals this metabolite is likely produced from the degradation of the methyl group in the β1 position by ω-oxidation, originating only from furan fatty acids with propyl in the position α2. This reaction is likely to occur in the liver, but perhaps it can also be carried out by the microbiota in the large intestine and suffers interference from the circadian cycle (Sinclair et al., 2018; Xu et al., 2017). Moreover, the detection of nonconjugated compounds in urine was previously reported as a result of active tubular secretion in the kidneys (Tsutsumi et al., 2002). Thus, we propose possible degradation pathways that gave rise to this metabolite, it may be the result of the gut microbiota action on furan fatty acids or it has been metabolized in the liver or kidneys. An analysis of the composition of F-acids in genipap juice would be interesting to better relate the presence of its metabolites in urine samples and its beneficial potential to human health. However, our results are encouraging in this regard.

Another biomarker, the cyclic dipeptide L,L-cyclo(leucylprolyl), exhibited significantly decreased excretion after medium-term consumption of genipap juice (Supplementary material Figure S4). L,L-Cyclo(leucylprolyl) was previously identified as a blood biomarker of consumption of white potatoes, corn, peas, alcohol and coffee (Guertin *et al*., 2014), exhibiting a significant increase after food ingestion. It was also reported as a urinary biomarker of coffee intake (Guertin *et al*., 2015; Rothwell *et al*., 2014). This compound appears to be a microbial derivative metabolite from microbiota (Yan *et al*., 2004), but in our case, ingestion of genipap juice appeared to reduce its excretion, which may be explained by a modification of microbiota or competition between substrates.

(Z)-3-(1-formyl-1-propenyl)pentanedioic acid, 3-(1-hydroxymethyl-1-propenyl)pentanedioic acid and 3-hexanoic acid are among the most affected metabolites, apart from iridoid derivatives, that appeared after medium-term consumption of genipap juice. These metabolites belong to the family of medium-chain fatty acids (MCFAs), which are substrates of energy metabolism and anabolic

processes in mammals. MCFAs arise mostly from dietary triglycerides, including those in milk and dairy products, are rich in carbon chains of 6-12 carbons and are easily absorbed into the bloodstream during digestion. MCFAs are metabolized into ketone bodies in the liver and can be converted to acetyl-CoA and used as a source of energy by the body (Lei *et al*., 2016; Wishart *et al*., 2013). Some studies have shown that MCFAs have health benefits, such as weight control (Aoyama, Nosaka, & Kasai, 2007; Takeuchi *et al*., 2008), the management of some neurological conditions (Lei *et al*., 2016) and antimicrobial activity (Huang *et al*., 2011). (Z)-3-(1-Formyl-1-propenyl)pentanedioic acid and 3-(1-hydroxymethyl-1-propenyl)pentanedioic acid were detected as biomarkers in urine only after nutritional intervention with genipap juice. Excretion of these MCFAs appeared to continue up to 24 h after the end of medium-term nutritional intervention, suggesting probable microbiota intermediation (Schönfeld & Wojtczak, 2016).

Vanillactic acid (VLA) is a catecholamine and an end-product of L-dopa metabolism (Manegold *et al*., 2009; Wassenberg *et al*., 2010). This metabolite that is normally present in urine increased only for some individual after medium-term intervention with genipap juice. VLA increase in urine has been related by some authors to aromatic L-amino acid decarboxylase (AADC) deficiency (Manegold *et al*., 2009; Wassenberg *et al*., 2010; Wassenberg *et al*., 2012), and AADC is an enzyme that produces serotonin and dopamine (Allen, Land, & Heales, 2009). This might support part of the health-related functions of the phytochemicals contained in genipap (Yanagihara *et al*., 2014). However, little has been reported in the literature about the real impact of elevated VLA levels in urine, and interpretation should be taken with caution.

Anyway, the following metabolites can be considered as good biomarkers of genipap juice intake, as assessed by the ROC curves (Table 1) : (Z)-3-(1-formyl-1-propenyl)pentanedioic acid, 12-demethylated-8-hydroxygenipinic acid, 3(4)-dehydrogenipic acid, 3(7)-dehydrogenipinic acid, 3(7)-dehydrogenipinic acid isomer, 11-deoxygenipic acid, dihydrocaffeic acid (DHCA), genipic acid glucuronide, 14-deoxygenipinic acid, genipic acid, CMPF, 11-deoxygenipic acid isomer, hydroxymethyl-cyclopenta[c]furan-1,3diol, hydroxyhydrocinnamic acid, 3-(1-hydroxymethyl-1-propenyl)pentanedioic acid, genipin isomer, nonate, dimethylphenol and 1-(4-hydroxyphenyl)-1,2-propanediol. A combined model could be also more robust than one that considered only one biomarker. For instance, a model combining urinary excretion of DHCA, 1-(4-hydroxyphenyl)-1,2-propanediol, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), vanillactic acid

(VLA), and 3-hexenoic acid is able to correctly assigned individuals in their respecting groups " before" and "after medium-term genipap consumption" with low error rate (<6%), AUC (0.995), confidence interval (CI=0.97-1) and permutation test values (n=1000; p<0.001) (Supplementary material Figure S2).

Conclusions

The main classes of metabolites excreted after medium-term consumption of genipap juice were iridoids, medium-chain fatty acids, hydroxycinnamic acids and CMPF, which have known biological activity. These compounds were highly correlated to genipap juice consumption, and when combined, they constitute a strong composite biomarker of genipap juice consumption, as shown by the ROC analysis.

Furthermore, these results provided an essential contribution to the knowledge about the bioavailability of bioactive compounds from genipap fruit and potential biomarkers of its intake. This study also gave some indication concerning health targets. Genipap shows potential in the development of functional foods or nutraceuticals, as we identified relevant phytoactive compounds linked to its consumption. The beneficial effects of genipap deserve more research, and the biomarkers of food intake listed in this paper could also be used in epidemiological studies to help understand the potential link between levels of genipap consumption and health status in Amazon regions.

Acknowledgments: We are grateful to all volunteers for participating in the study. We would like to thank Naturex, CNPq and ANRT for financial support.

Funding sources: This study was part of a collaborative project between a Brazilian (CNPq) and French (ANRT) institution, which is part of a thesis agreement entitled CIFRE Brazil and was mainly supported by the French company Naturex [CIFRE N° 2015/0001].

Conflicts of interest: none.

Author contributions: Conceptualization: LD, PFB, FV and HR; Data curation: LD, MT, LS and JCM; Formal analysis: LD, LS and JCM; Funding acquisition: PFB and HR; Investigation: LD;

Methodology: LD, L, JCM and HR; Project administration: LD, PFB, FV and HR; Supervision: PFB, FV and HR; Validation: PFB, JCM, FV and HR; Writing–original draft: LD; Writing–review and editing: LD, MT, LS, PFB, JCM, FV and HR.

Table 1: Putatively identified features. Table organized in ascending order according to the *p*-values obtained from t-tests.

Assigned identity	Molecular Formula	Theoretical *m/z*	Calculated *m/z*	Error(ppm)	RT(min)	Cluster Ions	*p*-value (T-test)	ROC curve AUC (95%CI)
3(4)-Dehydrogenipic acid	$C_9H_{14}O_4$	186.0884	$[M + H]^+$ 187.0962	-1.5	3.87		$2.579E^{-33}$	1.0 (1-1)
12-Demethylated-8-hydroxygenipinic acid	$C_{10}H_{12}O_7$	244.0588	$[M-H]^-$ 243.0510	0.0	1.82	244.0545 199.0613 155.0714	$9.7012E^{-44}$	1.0 (1-1)
(Z)-3-(1-Formyl-1-propenyl)pentanedioic acid	$C_9H_{12}O_5$	200.0684	$[M-H]^-$ 199.0613	0.4	4.06		$4.9379E^{-50}$	1.0 (1-1)
3(7)-Dehydrogenipinic acid isomer	$C_{11}H_{16}O_6$	244.0952	$[M-H]^-$ 243.0874	0.1	4.67		$4.7847E^{-37}$	0.999 (0.996-1)
3(7)-Dehydrogenipinic acid	$C_{11}H_{16}O_6$	244.0952	$[M-H]^-$ 243.0874	-0.2	4.05	225.0767	$7.2973E^{-51}$	0.999 (0.996-1)
14-Deoxygenipinic acid	$C_{11}H_{14}O_5$	226.0633	$[M + H]^+$ 227.0911	-0.7	4.68	163.0752 149.0596 135.0803	$4.7704E^{-23}$	0.998 (0.991-1)
Dihydroxyhydrocinnamic acid	$C_9H_{10}O_4$	182.0579	$[M + H]^+$ 183.0650	-1.0	4.83	137.0596	$1.5993E^{-24}$	0.997 (0.987-1)
Dihydroxyhydrocinnamic acid "isomer"	$C_9H_{10}O_4$	182.0579	$[M-H_2O]^+$ 165.0544	-0.8	4.06	137.0596	$8.6179E^{-30}$	0.994 (0.979- 1)
11-Deoxygenipic acid	$C_9H_{12}O_3$	168.0780	$[M + H]^+$ 169.0858	-1.0	3.89	151.0752 107.0853	$6.7696E^{-25}$	0.992 (0.979-1)
Hydroxyhydrocinnamic acid	$C_9H_{10}O_3$	166.0629	$[M + H]^+$ 167.0700	-0.7	1.79		$2.8069E^{-23}$	0.981 (0.943- 1)
11-Deoxygenipic acid isomer	$C_8H_8O_4$	168.0415	$[M + H]^+$ 169.0494	-0.3	4.15		$1.1632E^{-19}$	0.969 (0.924-0.994)
Genipic acid	$C_9H_{12}O_4$	184.0735	$[M + H]^+$ 185.0808	-0.0	5.38	186.0839 95.0853	$2.3564E^{-21}$	0.965 (0.911-0.998)
1-(4-Hydroxyphenyl)-1,2-propanediol	$C_9H_{12}O_3$	168.0786	$[M + H]^+$ 169.0858	-1.0	4.77		$9.08E^{-12}$	0.958 (0.916-0.987)
Hydroxymethyl-cyclopenta[c]furan-1,3diol	$C_8H_{12}O_4$	170.0586	171.0664	0.8	2.59		$2.7102E^{-17}$	0.953 (0.908-0.986)

Table 1. continued

Assigned identity	Molecular Formula	Theoretical *m/z*	Calculated *m/z*	Error(ppm)	RT(min)	Cluster Ions	*p*-value (T-test)	ROC curve AUC (95%CI)
3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF)	$C_{12}H_{16}O_5$	240.0997	$[M+H]^+$ 241.1068	-0.7	4.62	223.0962	$6.4083E^{-20}$	0.951 (0.91-0.986)
Dimethylphenol	$C_8H_{10}O$	122.0731	$[M+H]^+$ 123.0803	0.9	3.91		$7.99E^{-14}$	0.938 (0.877-0.971)
3-(1-Hydroxymethyl-1-propenyl)pentanedioic acid	$C_9H_{14}O_5$	202.0841	$[M-H]^-$ 201.0770	1.1	4.42		$1.6362E^{-16}$	0.917 (0.853-0.969)
Genipin isomer	$C_{11}H_{14}O_5$	226.0841	$[M+H]^+$ 227.09114	-0.9	4.04	209.08070 165.09090 163.07518 149.05965	$2.9639E^{-16}$	0.914 (0.847-0.966)
Nonate	$C_9H_{16}O_4$	188.1048	$[M-H]^-$ 187.0977	1.1	4.31		$5.45E^{-15}$	0.91 (0.844-0.965)
Cyclo(leucyl-prolyl)	$C_{11}H_{18}N_2O_2$	210.1368	$[M+H]^+$ 211.1438	-1.4	5.95		$1.37E^{-13}$	0.891 (0.806-0.943)
Prephenic acid	$C_{10}H_{10}O_6$	226.0477	$[M+H]^+$ 227.0549	-0.8	1.82	209.0443	$3.78E^{-12}$	0.877 (0.8-0.94)
Vanillactic acid (VLA)	$C_{10}H_{12}O_5$	212.0684	$[M-H]^-$ 211.0613	0.8	4.23		$3.35E^{-14}$	0.875 (0.788-0.942)
(1R,6R)-6-Hydroxy-2-succinylcyclohexa-2,4-diene-1-carboxylate	$C_{11}H_{12}O_6$	238.0488	$[M+H]^+$ 241.0703	-1.3	4.93	223.0598	$8.06E^{-11}$	0.865 (0.787-0.925)
Genipic acid isomer	$C_9H_{12}O_4$	184.0735	$[M-H]^-$ 183.0664	0.2	6.39		$2.52E^{-10}$	0.856 (0.767-0.926)

Table 1. continued

Assigned identity	Molecular Formula	Theoretical *m/z*	Calculated *m/z*	Error(ppm)	RT(min)	Cluster Ions	*p*-value (T-test)	ROC curve

							AUC (95%CI)
Methylxanthine	$C_6H_6N_4O_2$	166.0490	$[M-H]^-$ 165.0418	0.1	3.24	$1.98E^{-07}$	0.823 (0.727-0.906)
2-isopropylmalic acid	$C_7H_{12}O_5$	176.0684	$[M-H]^-$ 175.0613	0.9	4.87	$1.91E^{-06}$	0.771 (0.674-0.859)
Pimelic acid	$C_7H_{12}O_4$	160.0735	$[M-H]^-$ 159.0663	0.8	5.17	$1.88E^{-05}$	0.75 (0.656-0.837)
AFMU	$C_8H_{10}N_4O_4$	226.0702	$[M-H]^-$ 225.0630	0.8	1.43	$2.13E^{-05}$	0.75 (0.644-0.839)
3-Hexenoic acid	$C_6H_{10}O_2$	114.0680	$[M-H]^-$ 113.0608	1.0	4.86	$3.20E^{-05}$	0.737 (0.631-0.841)
Genipic acid glucuronide	$C_{15}H_{20}O_{10}$	360.1064	$[M + anydroGlu]^+$ 378.1391	0.8	4.79	$3.6732E^{-28}$	0.57 (0.446-0.671)

References

Allen, F., Pon, A., Wilson, M., Greiner, R., & Wishart, D. (2014). CFM-ID: a web server for annotation, spectrum prediction and metabolite identification from tandem mass spectra. *Nucleic Acids Research*, *42*(Web Server issue), W94–W99. https://doi.org/10.1093/nar/gku436

Allen, G. F. G., Land, J. M., & Heales, S. J. R. (2009). A new perspective on the treatment of aromatic l-amino acid decarboxylase deficiency. *Molecular Genetics and Metabolism*, *97*(1), 6–14. https://doi.org/10.1016/j.ymgme.2009.01.010

Aoyama, T., Nosaka, N., & Kasai, M. (2007). *Research on the nutritional characteristics of medium-chain fatty acids*. (54), 3–4.

Bentes, A. de S., & Mercadante, A. Z. (2014). Influence of the Stage of Ripeness on the Composition of Iridoids and Phenolic Compounds in Genipap (Genipa americana L.). *Journal of Agricultural and Food Chemistry*, *62*(44), 10800–10808. https://doi.org/10.1021/jf503378k

Caron, C., Duperier, C., Jacob, D., Thévenot, E. A., Giacomoni, F., Le Corguillé, G., … Goulitquer, S. (2014). Workflow4Metabolomics: A collaborative research infrastructure for computational metabolomics. *Bioinformatics*, *31*(9), 1493–1495. https://doi.org/10.1093/bioinformatics/btu813

Caspi, R., Billington, R., Ferrer, L., Foerster, H., Fulcher, C. A., Keseler, I. M., … Karp, P. D. (2016). The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Research*, *44*(D1), D471–D480. https://doi.org/10.1093/nar/gkv1164

Conceição, A., Helena Rossi, M., Oliveira, F., Takser, L., & Lafond, J. (2011). *Genipa americana (Rubiaceae) Fruit Extract Affects Mitogen-Activated Protein Kinase Cell Pathways in Human Trophoblast–Derived BeWo Cells: Implications for Placental Development* (Vol. 14). https://doi.org/10.1089/jmf.2009.0279

de Souza, V. R., Pereira, P. A. P., Queiroz, F., Borges, S. V., & de Deus Souza Carneiro, J. (2012). Determination of bioactive compounds, antioxidant activity and chemical composition of Cerrado Brazilian fruits. *Food Chemistry*, *134*(1), 381–386. https://doi.org/10.1016/j.foodchem.2012.02.191

Degtyarenko, K., de Matos, P., Ennis, M., Hastings, J., Zbinden, M., McNaught, A., … Ashburner, M. (2008). ChEBI: a database and ontology for chemical entities of biological interest. *Nucleic Acids Research*, *36*(Database issue), D344–D350. https://doi.org/10.1093/nar/gkm791

Dickson, L., Tenon, M., Svilar, L., Fança-Berthon, P., Lugan, R., Martin, J.-C., … Rogez, H. (2018). Main Human Urinary Metabolites after Genipap (*Genipa americana* L.) Juice Intake. *Nutrients*, *10*(9). https://doi.org/10.3390/nu10091155

Espín, J. C., González-Sarrías, A., & Tomás-Barberán, F. A. (2017). The gut microbiota: A key factor in the therapeutic effects of (poly)phenols. *The 5th International Conference on the Mechanism of Action of Nutraceuticals*, *139*, 82–93. https://doi.org/10.1016/j.bcp.2017.04.033

Figueiredo, R., Maia, G., Holanda, L., & Monteiro, J. (1986). *Caracteristicas fisicas e quimicas do genipapo*. *4*, 421–428. https://doi.org/10.3390/nu5104159

Gabriela Costa Lima Porto, R., Verônica Sousa Cardoso, B., Vanessa dos Anjos Barros, N., Mayara Ferreira Cunha, E., Antônio da Mota Araújo, M., & Moreira-Araújo, R. (2014). *Chemical Composition and Antioxidant Activity of Genipa Americana L. (Jenipapo) of the Brazilian Cerrado* (Vol. 3). https://doi.org/10.15640/jaes.v3n4a4

Garcia-Aloy, M. *et al.* Novel Multimetabolite Prediction of Walnut Consumption by a Urinary Biomarker Model in a Free-Living Population: the PREDIMED Study. *J. Proteome Res.* 13, 3476–3483 (2014).

Gerlich, M., & Neumann, S. (2013). MetFusion: Integration of compound identification strategies. *J Mass Spectrom*, *48*. https://doi.org/10.1002/jms.3123

Guertin, K. A., Loftfield, E., Boca, S. M., Sampson, J. N., Moore, S. C., Xiao, Q., … Sinha, R. (2015). Serum biomarkers of habitual coffee consumption may provide insight into the mechanism underlying the association between coffee consumption and colorectal cancer. *The American Journal of Clinical Nutrition*, *101*(5), 1000–1011. https://doi.org/10.3945/ajcn.114.096099

Guertin, K. A., Moore, S. C., Sampson, J. N., Huang, W.-Y., Xiao, Q., Stolzenberg-Solomon, R. Z., … Cross, A. J. (2014). Metabolomics in nutritional epidemiology: Identifying metabolites associated with diet and quantifying their potential to uncover diet-disease relations in populations. *The American Journal of Clinical Nutrition*, *100*(1), 208–217. https://doi.org/10.3945/ajcn.113.078758

Hamacek, F., Moreira, A., Martino, H., Ribeiro, S., & Pinheiro-Sant'Ana, H. (2013). *Valor nutricional, caracterização física e físico-química de genipapo (Genipa americana L.) do Cerrado de Minas Gerais*. *24*.

Hanhineva, K., Lankinen, M. A., Pedret, A., Schwab, U., Kolehmainen, M., Paananen, J., … Mykkänen, H. (2015). Nontargeted Metabolite Profiling Discriminates Diet-Specific Biomarkers for Consumption of Whole Grains, Fatty Fish, and Bilberries in a Randomized Controlled Trial. *The Journal of Nutrition*, *145*(1), 7–17. https://doi.org/10.3945/jn.114.196840

Hansen, D. de S., Silva, S. A., Fonseca, A. A. O., Hansen, O. A. de S., & FranÃ\Sa, N. O. (2008). Caracterização química de frutos de genipapeiros nativos do Recôncavo Baiano visando ao consumo natural e industrialização. *Revista Brasileira de Fruticultura*, *30*, 964–969.

Huang, C. B., Alimova, Y., Myers, T. M., & Ebersole, J. L. (2011). Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. *Archives of oral biology*, *56*(7), 650–654. https://doi.org/10.1016/j.archoralbio.2011.01.011

Huang, J., de Paulis, T., & May, J. M. (2004). Antioxidant effects of dihydrocaffeic acid in human EA. hy926 endothelial cells. *The Journal of Nutritional Biochemistry*, *15*(12), 722–729. https://doi.org/10.1016/j.jnutbio.2004.07.002

Hwa, J. S., Mun, L., Kim, H. J., Seo, H. G., Lee, J. H., Kwak, J. H., … Chang, K. C. (2011). Genipin Selectively Inhibits TNF-α-activated VCAM-1 But Not ICAM-1 Expression by Upregulation of PPAR-γ in Human Endothelial Cells. *The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology*, *15*(3), 157–162. https://doi.org/10.4196/kjpp.2011.15.3.157

Kanehisa, M., & Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*, *28*. https://doi.org/10.1093/nar/28.1.27

Koo, H.-J., Song, Y. S., Kim, H.-J., Lee, Y.-H., Hong, S.-M., Kim, S.-J., … Park, E.-H. (2004). Antiinflammatory effects of genipin, an active principle of gardenia. *European Journal of Pharmacology*, *495*(2), 201–208. https://doi.org/10.1016/j.ejphar.2004.05.031

Lei, E., Vacy, K., & Boon, W. C. (2016). Fatty acids and their therapeutic potential in neurological disorders. *Special Issue: Neuro-Nutraceuticals: Further Insights into their Promise for Brain Health*, *95*, 75–84. https://doi.org/10.1016/j.neuint.2016.02.014

Li, Z., Zhang, T.-B., Jia, D.-H., Sun, W.-Q., Wang, C.-L., Gu, A.-Z., & Yang, X.-M. (2018). Genipin inhibits the growth of human bladder cancer cells via inactivation of PI3K/Akt signaling. *Oncology Letters*, *15*(2), 2619–2624. https://doi.org/10.3892/ol.2017.7588

Liu, J., Yin, F., Guo, L., Deng, X., & Hu, Y. (2009). Neuroprotection of geniposide against hydrogen peroxide induced PC12 cells injury: Involvement of PI3 kinase signal pathway. *Acta Pharmacologica Sinica*, *30*(2), 159–165. https://doi.org/10.1038/aps.2008.25

Manegold, C., Hoffmann, G. F., Degen, I., Ikonomidou, H., Knust, A., Laaß, M. W., … Hörster, F. (2009). Aromatic l-amino acid decarboxylase deficiency: Clinical features, drug therapy and follow-up. *Journal of Inherited Metabolic Disease*, *32*(3), 371–380. https://doi.org/10.1007/s10545-009-1076-1

Mikami Masaki, & Takikawa Hajime. (2008). Effect of genipin on the biliary excretion of cholephilic compounds in rats. *Hepatology Research*, *38*(6), 614–621. https://doi.org/10.1111/j.1872-034X.2007.00309.x

Morton, J. (1987). Genipap. In *Fruits of warm climates* (p. 441–443). Miami, FL.

Morton, J. F. ([s.d.]). Genipap. In *Fruits of warm climates*.

Pacheco, P., da Paz, J. G., da Silva, C. O., & Pascoal, G. B. (2015). Composição centesimal, compostos bioativos e parâmetros físico-químicos do genipapo (*Genipa americana* L.) in natura. *DEMETRA: Alimentação, Nutrição & Saúde; v. 9, n. 4 (2014)*. https://doi.org/10.12957/demetra.2014.11310

Persicke, M., Rückert, C., Plassmeier, J., Jonathan Stutz, L., Kessler, N., Kalinowski, J., … Neuweger, H. (2012). *MSEA: Metabolite set enrichment analysis in the MeltDB metabolomics software platform: Metabolic profiling of Corynebacterium glutamicum as an example* (Vol. 8). https://doi.org/10.1007/s11306-011-0311-6

R Core Team. (2013). *R: A language and environment for statistical computing*. Recuperado de http://www.R-project.org/.

Rogez, H., Buxant, R., Mignolet, E., Souza, J. N. S., Silva, E. M., & Larondelle, Y. (2004). Chemical composition of the pulp of three typical Amazonian fruits: Araça-boi (Eugenia stipitata), bacuri (Platonia insignis) and cupuaçu (Theobroma grandiflorum). *European Food Research and Technology*, *218*(4), 380–384. https://doi.org/10.1007/s00217-003-0853-6

Rothwell, J. A., Fillâtre, Y., Martin, J.-F., Lyan, B., Pujos-Guillot, E., Fezeu, L., … Manach, C. (2014). New Biomarkers of Coffee Consumption Identified by the Non-Targeted Metabolomic Profiling of Cohort Study Subjects. *PLoS ONE*, *9*(4), e93474. https://doi.org/10.1371/journal.pone.0093474

Rufino, M. do S. M., Alves, R. E., de Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, *121*(4), 996–1002. https://doi.org/10.1016/j.foodchem.2010.01.037

Ruttkies, C., Schymanski, E. L., Wolf, S., Hollender, J., & Neumann, S. (2016). MetFrag relaunched: Incorporating strategies beyond in silico fragmentation. *Journal of Cheminformatics*, *8*(1), 3. https://doi.org/10.1186/s13321-016-0115-9

Schönfeld, P., & Wojtczak, L. (2016). Short- and medium-chain fatty acids in energy metabolism: The cellular perspective. *Journal of Lipid Research*, *57*(6), 943–954. https://doi.org/10.1194/jlr.R067629

Shanmugam, M. K., Shen, H., Tang, F. R., Arfuso, F., Rajesh, M., Wang, L., … Sethi, G. (2018). Potential role of genipin in cancer therapy. *Pharmacological Research*, *133*, 195–200. https://doi.org/10.1016/j.phrs.2018.05.007

Silva, A. V. C. da, Yaguiu, P., Almeida, C. S., & Feitosa, R. B. (2009). *Caracterização físico-química de genipapo*. Embrapa.

Sinclair, A. J., Xu, L., & Wang, Y. (2018). 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF): A metabolite identified after consumption of fish oil and fish. *Nutrition Bulletin*, *43*(2), 153–157. https://doi.org/10.1111/nbu.12321

Smith, C. A., O'Maille, G., Want, E. J., Qin, C., Trauger, S. A., Brandon, T. R., … Siuzdak, G. (2005). METLIN: a metabolite mass spectral database. *Ther Drug Monit*, *27*, 747–751.

Smith, Colin A., Want, E. J., O'Maille, G., Abagyan, R., & Siuzdak, G. (2006). XCMS: Processing Mass Spectrometry Data for Metabolite Profiling Using Nonlinear Peak Alignment, Matching, and Identification. *Analytical Chemistry*, *78*(3), 779–787. https://doi.org/10.1021/ac051437y

Son, M., Lee, M., Ryu, E., Moon, A., Jeong, C.-S., Jung, Y. W., … Kang, H. (2015). Genipin as a novel chemical activator of EBV lytic cycle. *Journal of Microbiology*, *53*(2), 155–165. https://doi.org/10.1007/s12275-015-4672-9

Sumner, L. W., Amberg, A., Barrett, D., Beale, M. H., Beger, R., Daykin, C. A., … Viant, M. R. (2007). Proposed minimum reporting standards for chemical analysis. *Metabolomics*, *3*(3), 211–221. https://doi.org/10.1007/s11306-007-0082-2

Takeuchi, H., Sekine, S., Kojima, K., & Aoyama, T. (2008). *The application of medium-chain fatty acids: Edible oil with a suppressing effect on body fat accumulation*. (17).

Tsutsumi, Y., Deguchi, T., Takano, M., Takadate, A., Lindup, W. E., & Otagiri, M. (2002). Renal Disposition of a Furan Dicarboxylic Acid and Other Uremic Toxins in the Rat. *Journal of Pharmacology and Experimental Therapeutics*, *303*(2), 880. https://doi.org/10.1124/jpet.303.2.880

Viljoen, A., Mncwangi, N., & Vermaak, I. (2012). *Anti-Inflammatory Iridoids of Botanical Origin* (Vol. 19). https://doi.org/10.2174/092986712800229005

Villachica, H., de Carvalho, J. E. U., Mercado, R. S., Izquierdo, J., Egg, A. B., Amazónica, S. pro tempore del T. de C., … Nations, A. O. of the U. (1996). *Frutales y hortalizas promisorios de la Amazonía*. Recuperado de https://books.google.fr/books?id=ocW5HAAACAAJ

Wassenberg, T., Willemsen, M. A. A. P., Geurtz, P. B. H., Lammens, M., Verrijp, K., Wilmer, M., … Verbeek, M. M. (2010). Urinary dopamine in aromatic L-amino acid decarboxylase deficiency: The unsolved paradox. *Molecular Genetics and Metabolism*, *101*(4), 349–356. https://doi.org/10.1016/j.ymgme.2010.08.003

Wassenberg, Tessa, Monnens, L. A. H., Geurtz, B. P. B. H., Wevers, R. A., Verbeek, M. M., & Willemsen, M. A. A. P. (2012). The Paradox of Hyperdopaminuria in Aromatic l-Amino Acid Deficiency Explained. In *JIMD Reports—Case and Research Reports, 2012/1* (p. 39–45). https://doi.org/10.1007/8904_2011_84

Wishart, D. S., Jewison, T., Guo, A. C., Wilson, M., Knox, C., Liu, Y., … Scalbert, A. (2013). HMDB 3.0—The Human Metabolome Database in 2013. *Nucleic Acids Research*, *41*(D1), D801–D807. https://doi.org/10.1093/nar/gks1065

Wittemer, S. M., Ploch, M., Windeck, T., Müller, S. C., Drewelow, B., Derendorf, H., & Veit, M. (2005). Bioavailability and pharmacokinetics of caffeoylquinic acids and flavonoids after oral administration of Artichoke leaf extracts in humans. *Phytomedicine*, *12*(1), 28–38. https://doi.org/10.1016/j.phymed.2003.11.002

Wu, S., Wang, G., Liu, Z., Rao, J., Lü, L., Xu, W., … Zhang, J. (2009). Effect of geniposide, a hypoglycemic glucoside, on hepatic regulating enzymes in diabetic mice induced by a high-fat diet and streptozotocin. *Acta Pharmacologica Sinica*, *30*(2), 202–208. https://doi.org/10.1038/aps.2008.17

Xia, J. (Jeff, & S Wishart, D. (2011). *Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst* (Vol. 6). https://doi.org/10.1038/nprot.2011.319

Xu, L., Sinclair, A.J., Faiza, M., Li, D., Han, X., Yin, H., Wang, Y., 2017. Furan fatty acids – Beneficial or harmful to health? Progress in Lipid Research 68, 119–137. https://doi.org/10.1016/j.plipres.2017.10.002

Yan, P.-S., Song, Y., Sakuno, E., Nakajima, H., Nakagawa, H., & Yabe, K. (2004). Cyclo(l-Leucyl-l-Prolyl) Produced by Achromobacter xylosoxidans Inhibits Aflatoxin Production by Aspergillus parasiticus. *Applied and Environmental Microbiology*, *70*(12), 7466–7473. https://doi.org/10.1128/AEM.70.12.7466-7473.2004

Yanagihara, N., Zhang, H., Toyohira, Y., Takahashi, K., Ueno, S., Tsutsui, M., & Takahashi, K. (2014). New Insights Into the Pharmacological Potential of Plant Flavonoids in the Catecholamine System. *Journal of Pharmacological Sciences*, *124*(2), 123–128. https://doi.org/10.1254/jphs.13R17CP

Figure Captions

Figure 1: PLS-DA metabolomic profiling of urine samples. (A) The PLS-DA loading plot shows strong discrimination between groups; (B) model validation assessed by permutation test (n=100) values of R2Y (0.85) and Q2Y (-0.0931;0,78). The *p*-value after CV-ANOVA = 2.36 e^{28}.

Figure 2: Venn diagram of the thirty-nine identified metabolites observed after acute and medium-term consumption of genipap juice.

Figure 3: Urinary excretion of genipic acid and 12-demethylated-8-hydroxygenipinic acid (D8H genipinic acid) for 16 individuals after 3 weeks daily ingestion of genipap juice (time series correspond to urine collected 6, 12 and 24 hours).

CONFLICT OF INTERESTS

All authors have approved the manuscript and agree that it should be submitted to "***Food Research International***". My co-authors and I declare no conflict of interest. The authors also assure that no part of this work is being considered for publication by another journal and this paper has not been published before in any form.

I declare that the present study was conducted in strict accordance with the international ethical guidelines for research involving humans established in the Declaration of Helsinki and in compliance with resolution 196/96 - of the National Health Council of the Ministry of Health from Brazil CNS/MS. The study was approved by the ethical committee of the Institute of Health Sciences of the Federal University of Para (Belém, Brazil; ethical ID: 1.436.134).

I look forward to hearing from you and remain,

Pr. Fabrice Vaillant

CREDIT AUTHOR STATEMENT

Conceptualization: LD, PFB, HR and FV

Methodology: LD, LS, JCM, HR and FV

Validation: PFB, JCM, HR and FV

Formal analysis: LD, LS, FV and JCM

Investigation: LD

Resources: PFB, HR and FV

Data curation: LD, MT, LS and JCM

Writing – original draft: LD

Writing – review & editing: LD, MT, LS, PFB, JCM, HR and FV

Visualization: LD and FV

Supervision: PFB, HR and FV

Project administration: LD, PFB, HR and FV

Funding acquisition: PFB, HR and FV

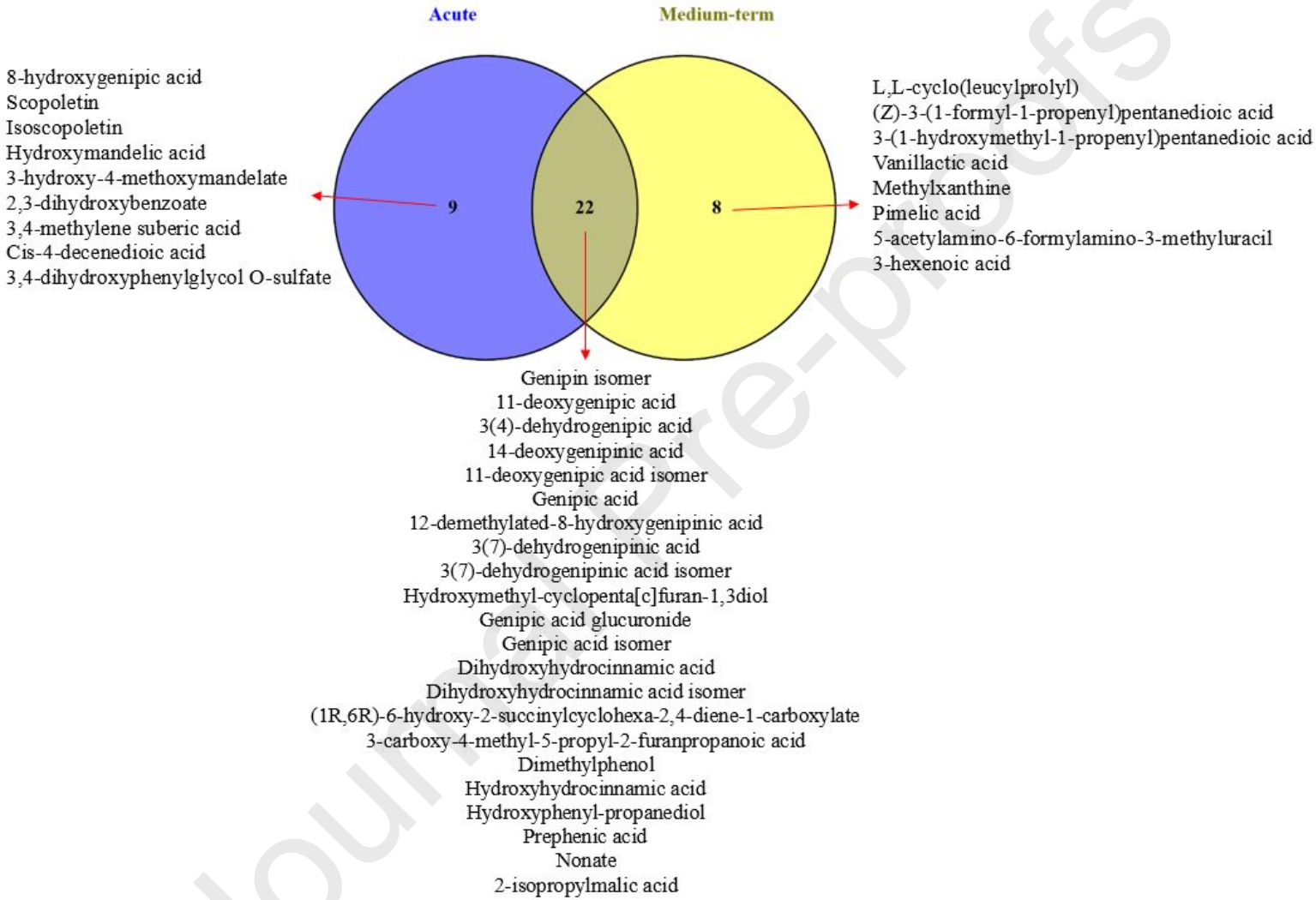
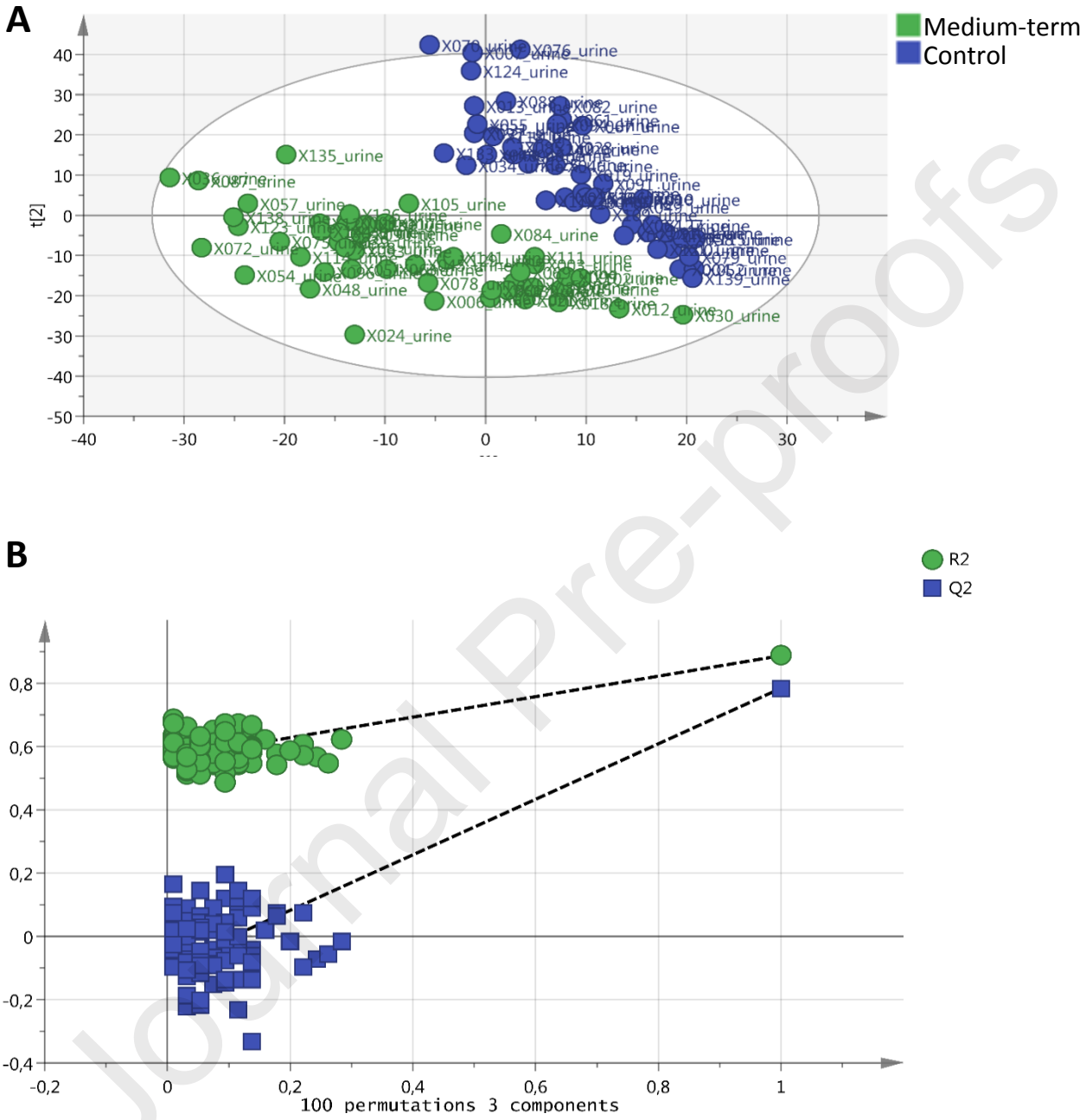


Figure 1:

Figure 2



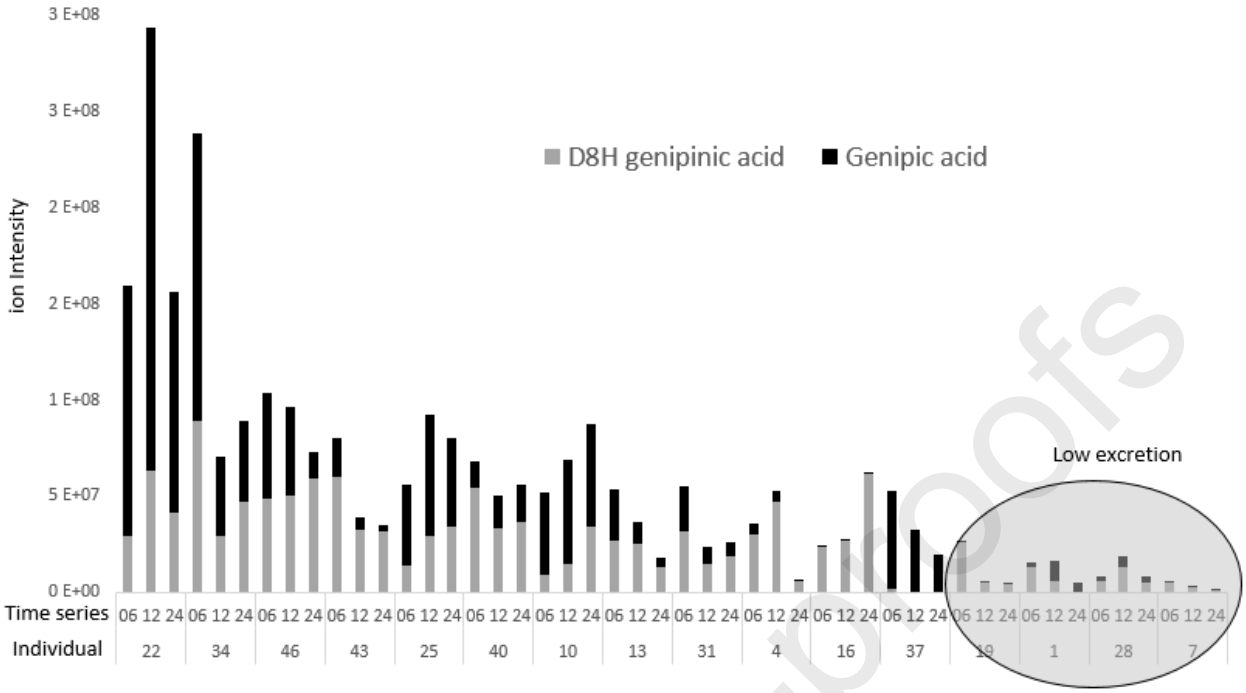
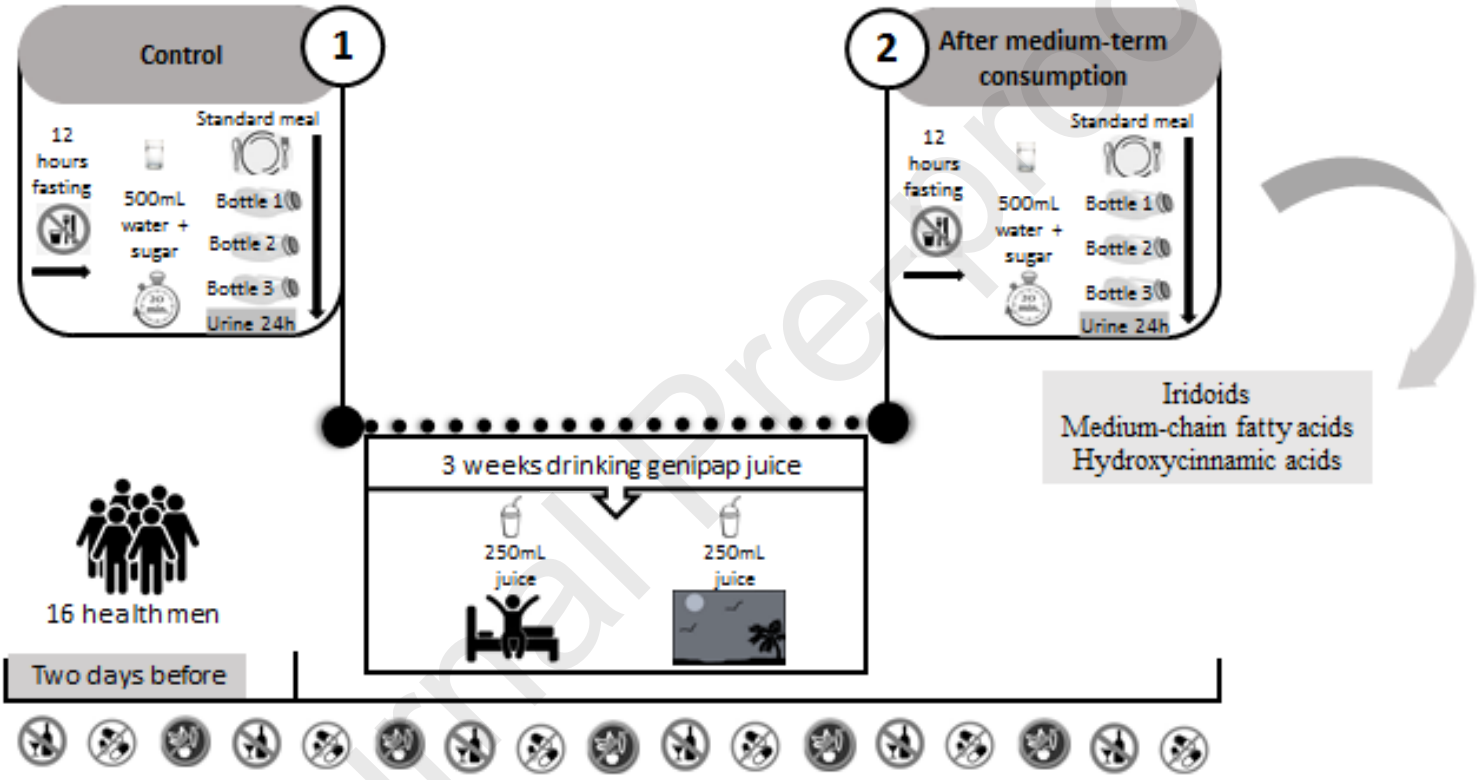


Figure 3:



Highlights

- Genipap juice is a source of bioactive compounds.
- The iridoids, medium-chain fatty acids, and hydroxycinnamic acids are the main metabolites of genipap juice intake in medium-term.
- DHCA, 1-(4-hydroxyphenyl)-1,2-propanediol and 3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) were established as biomarkers of genipap consumption.
- Genipap juice intake can be used in epidemiological studies to help understand it use and effect to treat some diseases.