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Original Article

Analysis of potential anti-aging beverage Pru, a traditional Cuban refreshment, by desorption electrospray ionization-mass spectrometry and FTICR tandem mass spectrometry



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ABSTRACT

Aging has been established as a major risk factor for prevalent diseases and hence, the development of anti-aging medicines is of great importance. Recently, herbal fermented beverages have emerged as a promising source of potential anti-aging drug. Pru, a traditional Cuban refreshment produced by decoction and fermentation of multispecies plants with sugar, has been consumed for many years and is claimed to have multiple medicinal properties. Besides the traditional method, Pru is also manufactured industrially. The present study analyzed the major components of both traditional Pru (TP) and industrial Pru (IP) to reveal their potential application in promoting the health span. We performed desorption electrospray ionization-mass spectrometry (DESI-MS) and acquired mass spectra by scanning over the 50–1200 *m/z* range in both positive and negative ion modes. Fourier transform ion cyclotron resonance (FTICR) tandem mass spectrometry (MS/MS) was performed for validating the compound assignments. Three important compounds were identified by comparing the MS and MS/MS spectra with reported literature and the online database. One of the identified compounds, gluconic acid, was found to be the most abundant shared metabolite between TP and IP whereas the other two compounds, magnoflorine and levan were exclusively detected in TP. The present study is the first report of

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component profiling in Cuban traditional and industrial Pru using DESI-MS and FTICR MS/MS, and reveals the potential application of Pru as a health-promoting agent.

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1. Introduction

Aging, a complex process characterized by successive loss of physiological integrity and increased vulnerability to death, has been associated with increased risk of diseases such as cardiovascular disorders, neurodegenerative diseases, diabetes mellitus and cancer [1]. An aging society has already arrived and the percentage of the world population over 60 years is estimated to double from approximately 11% to 22% between 2000 and 2050 [2]. Therefore, the development of anti-aging medicine for maintaining a healthy life by preventing and/or postponing the aging process is of great importance.

Throughout the ages, herbal fermented beverages have been consumed worldwide and are claimed to have medicinal properties [3]. Recently, scientific interest in these preparations has expanded remarkably because plant secondary metabolites [4] and microbial metabolites [5] exhibit numerous beneficial health properties that result in prolonged health span. For example, oral supplementation with the natural polyamine spermidine extends lifespan by reducing cardiac hypertrophy and preserving diastolic function in old mice [6]. Natural polyphenolic compounds are also known to inhibit and/or delay the production of free radicals and lipid peroxidation through antioxidant properties thereby potentially preventing prevalent diseases such as cardiovascular disorders [7] and neurodegenerative diseases [8].

Pru, popularly known as Pru Oriental, is a traditional Cuban refreshment and medicinal beverage that is produced by decoction and fermentation of multispecies plants, mainly *Gouania polygama*, *Smilax domingensis* and *Pimenta dioica* with cane sugar [9]. Its origin is traced back to the 1800s, the period after Haitian Revolution, when French settlers migrated to eastern Cuba bringing with them their customs and traditions, including Pru oriental [9]. Throughout history, Pru has been believed to possess antihypertensive properties, and many people with hypertension consume it daily to reduce blood pressure [9]. It is also claimed to have stomachic, depurative and diuretic properties [9]. Thus, Pru represents a promising link with improved health outcomes. Besides the traditional method, Pru is also produced industrially nowadays. To date, biochemical analysis of components in both traditional and industrial Pru has not been described, which hinders the scientific validation of its claimed health effects.

In the last few years, mass spectrometry (MS) has been employed extensively in metabolomics for the direct visualization of biomolecules [10] using a number of tissue samples [11,12] as well as single cells [13]. Recently, desorption electrospray ionization-mass spectrometry (DESI-MS) has emerged as a powerful tool for detecting small molecules due

to its soft ionization technique and matrix-free simple workflow [14]. Even though DESI-MS has developed primarily for the analysis of solid samples, capability for analyzing liquid samples has also been established as its other strength [15]. DESI uses charged microdroplets of solvent onto the sample surface where analytes are extracted, ionized, and desorbed into the mass spectrometer (Fig. 1). One of its major advantages, compared to other techniques, is that DESI does not require additional sample preparation, resulting in a simplified analytical procedure for rapid identification of chemicals in samples, all under ambient conditions [14]. In contrast, FTICR-MS is well known for its high-mass resolution and FTICR MS/MS is useful for identification of compounds [16]. In our current study, we employed DESI-MS and FTICR MS/MS to analyze the Cuban traditional Pru (TP) and industrial Pru (IP) to identify their major components.

2. Materials and methods

2.1. Samples

The Pru used in this work was produced in Cuba in the province Santiago de Cuba. TP was produced by a very old local producer from the mountain side and IP was purchased as a beverage from the industry. Both were obtained for this research by Theragnostic Laboratory, University Hospital Calixto Garcia, La Habana, Cuba, transported in an ice box and stored at 4 °C until use.

2.2. Chemicals

Methanol and ultrapure water were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sodium formate was obtained from Sigma–Aldrich (USA).

2.3. DESI-MS

Mass spectra were obtained from TP and IP ions in both positive and negative ion modes. All experiments were performed using a desorption electrospray ionization (DESI) source attached to a quadrupole time-of-flight (Q-TOF) mass spectrometer (Xevo G2-XS Q-TOF, Waters, Milford, MA, USA). The mass spectra were calibrated externally using 500 μ M sodium formate in 2-propanol:water (90:10, v/v), prior to measurement.

Liquid droplets (0.3 μ L) of samples were placed on a glass slide (Matsunami, Japan) and allowed to dry (Fig. 1). Individual glass slides were used for each sample and experiments were carried out separately to avoid cross-contamination. The spray solvent (methanol:water, 98:2, v/v) was delivered at a

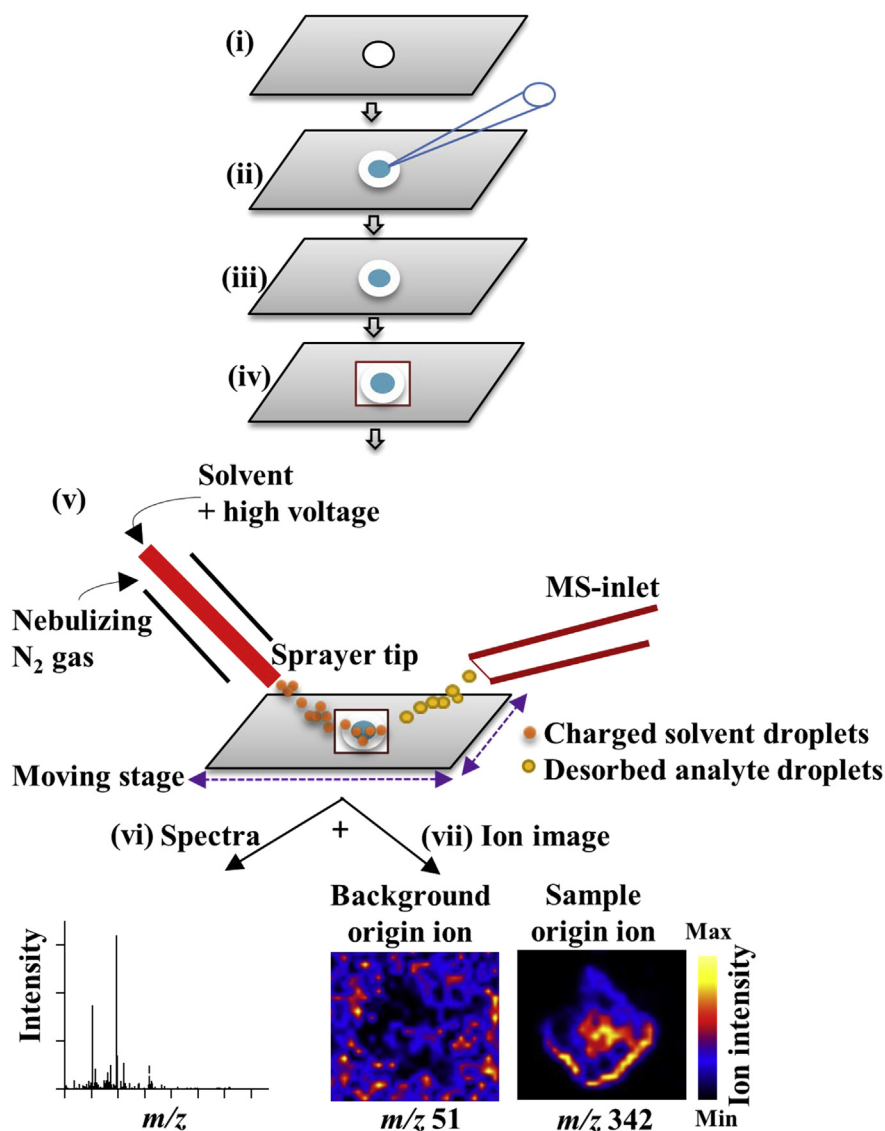


Fig. 1 – DESI-MS workflow for Pru analysis. (i) Acquisition of an optical image of a glass slide marked with a circle in the center. (ii) Application of sample droplet within the circle and (iii) air-dried. (iv) Defining the measurement area centering the droplet (droplet area $\sim 2.5 \text{ mm}^2$ plus surroundings). (v) DESI-MS events, (vi) mass spectra, and (vii) two representative ion images of TP showing the distinctions between background and sample area ions.

flow rate of $2 \mu\text{L}/\text{min}$ using a solvent pump (ACQUITY UPLC Binary Solvent Manager, Waters, Milford, MA, USA). Mass resolution, mass window, and collision energy were set at 20,000, 0.02, and 4.00 (manufacturer's unit), respectively. Ions from an area of about 7 mm^2 (droplet area $\sim 2.5 \text{ mm}^2$ plus surroundings) centering the sample droplet were obtained in a mass range of m/z 50–1200. A software-controlled 2D moving stage was used to scan the defined area with a scan rate and pixel size of $400 \mu\text{m}/\text{s}$ and $200 \mu\text{m} \times 200 \mu\text{m}$, respectively. The spray voltage was set at $+4.0 \text{ kV}$ for the positive ion mode and -4.0 kV for the negative ion mode, and the nebulizing nitrogen gas was delivered at 0.4 MPa from an external gas cylinder. An ion transfer capillary (mass inlet) temperature of $120 \text{ }^\circ\text{C}$ and a spray impact angle of 80° was used. The emitter was exposed from the sprayer tip by $\sim 0.5 \text{ mm}$. The emitter tip-to-surface, emitter tip-to-mass inlet, and mass inlet-to-surface

distances were $\sim 2 \text{ mm}$, $\sim 6 \text{ mm}$ and $\sim 0.5 \text{ mm}$, respectively. All experiments were repeated at least thrice under the identical conditions to assess the reproducibility of the spectra.

2.4. ESI FTICR MS/MS

MS/MS experiments for the selected m/z values were performed using an FTICR mass spectrometer (Bruker Solarix XR) equipped with a superconducting magnet (Bruker Daltonics, Germany). An fmsControl (Bruker Daltonics, Germany) software was used to control the equipment. The samples were directly infused at a flow rate of $120 \mu\text{L}/\text{h}$ into the ESI source. The ESI source conditions were optimized as follows: a nebulizer gas pressure of 1.0 bar , a dry gas ($180 \text{ }^\circ\text{C}$) flow rate of $4.0 \text{ L}/\text{min}$, an ion flight time of 1.0 ms , an ion accumulation time of 3 s and an end plate offset of -500 V .

The capillary voltage was set at 4.5 kV for the positive ion mode and 3.9 kV for the negative ion mode. Each spectrum was acquired by accumulating 5 scans of time domain transient signals in 64 kilo-point time-domain data sets. To avoid cross-contamination, the infusion line was washed using 100% methanol between sample runs. For MS/MS experiments, continuous accumulation of selected ions (CASI)-quadrupole collision-induced dissociation (qCID) mode was selected. Argon was used as the collision gas, and collision energy was adjusted from 0 V to 25 V. Collision RF amplitude, RF frequency, and isolation windows were set as 1200 Vpp, 2 MHz, and 10 m/z , respectively. Prior to measurement, the mass spectra were calibrated externally using sodium formate solution (1 mg/mL in 50% methanol).

2.5. Data analysis

Ion images constructed using HDImaging software (Waters) were used for peak picking from the 1000 most intense peaks of each mass spectra. Discernible images exclusively observed in droplet area (the central area of the defined area during acquisition) were considered to be originated from the sample (Fig. 1, Supplementary Fig. 1), and the m/z values corresponding to these images were listed. Peak list and the corresponding average intensity values were extracted from the droplet area and then spectra were reconstructed using MS Excel. Identification of compounds was performed by precisely matching the MS and MS/MS spectra with previously reported data and free online databases, including Metlin (https://metlin.scripps.edu/landing_page.php?pgcontent=simple_search).

3. Results

3.1. DESI-MS analysis of Pru

We performed DESI-MS on TP and IP, and acquired mass spectra by scanning over the m/z range of 50–1200 in both positive and negative ion modes. Both ionization modes contributed greatly with important ions to characterize the major components of the beverage under study. Ions which

were subsequently identified by FTICR MS/MS analyses were listed in Table 1.

Fig. 2(a) shows the representative mass spectra obtained from TP and IP in positive ion mode, where a distinct ion of m/z 342 was exclusively detected in the spectra of TP and was tentatively assigned as magnoflorine. Another important ion exclusively observed in TP in the positive ion mode was of m/z 527, which was proposed as levan.

As seen in Fig. 2(b), the ion of m/z 195 was observed as the most intense ion in both TP and IP in the negative ion mode, and was proposed as gluconic acid. Interestingly, the abundance of this ion is approximately 1.5 times higher in TP compared to that in IP. We also listed a number of other ions with considerable intensity which are shown in Table 2.

3.2. ESI FTICR MS/MS analysis of Pru

For validation of the tentatively assigned compounds, FTICR MS/MS analyses were performed and the observed spectra were compared with the fragmentation patterns of molecules described in literature and in the online database.

The MS/MS spectra of the ion at m/z 195 showed fragment ions at m/z 177, m/z 159 and m/z 129 (Fig. 3(a), Table 1). These are characteristic fragment ions of gluconic acid [17], hence identified.

When the ion at m/z 342 was subjected to MS/MS analysis, it produced prominent fragment ions at m/z 265, m/z 297 and m/z 282 (Fig. 3(b), Table 1), and was finally assigned as magnoflorine based on previous reports [18,19].

In MS/MS analysis of the ion at m/z 527, we observed product ions at m/z 365 and m/z 347 (Fig. 3(c), Table 1), which are characteristic fragment ions of levan [20].

The expected ion of sucrose at m/z 365 showed a prominent fragment ion at m/z 203 (Fig. 3(d), Table 1), which is a well-known product ion of this compound [21].

A number of other important ions observed in DESI-MS spectra with considerable intensity were also subjected to MS/MS analysis and most of them successfully represented their fragment ions (Table 2). However, these ions were not assigned due to lack of information in literature as well as in the online database.

Table 1 – FTICR MS/MS data obtained from TP and IP along with molecular assignments.

m/z observed	TP	IP	m/z calculated	Error (ppm)	MS/MS fragments (observed)	MS/MS fragments (reported)	Molecular assignments	Molecular formula
Positive ion								
342.1692	○	×	342.1705	4	265.08, 297.11, 282.08	297.11, 265.08, 282.08 [18,19]	Magnoflorine	$C_{20}H_{24}NO_4^-$
365.1055	○	○	365.1054	0	203.05	203.05 [21]	Sucrose	$C_{12}H_{22}O_{11}$
527.1590	○	×	527.1583	1	365.10, 347.09	365.1, 347.09 [20]	Levan	$C_{18}H_{32}O_{16}$
Negative ion								
195.0518	○	○	195.0510	3	177.04, 159.03, 129.01	177.04, 159.03, 129.01, 75.0 [17]	Gluconic acid	$C_6H_{12}O_7$
○: Observed; ×: Not observed.								

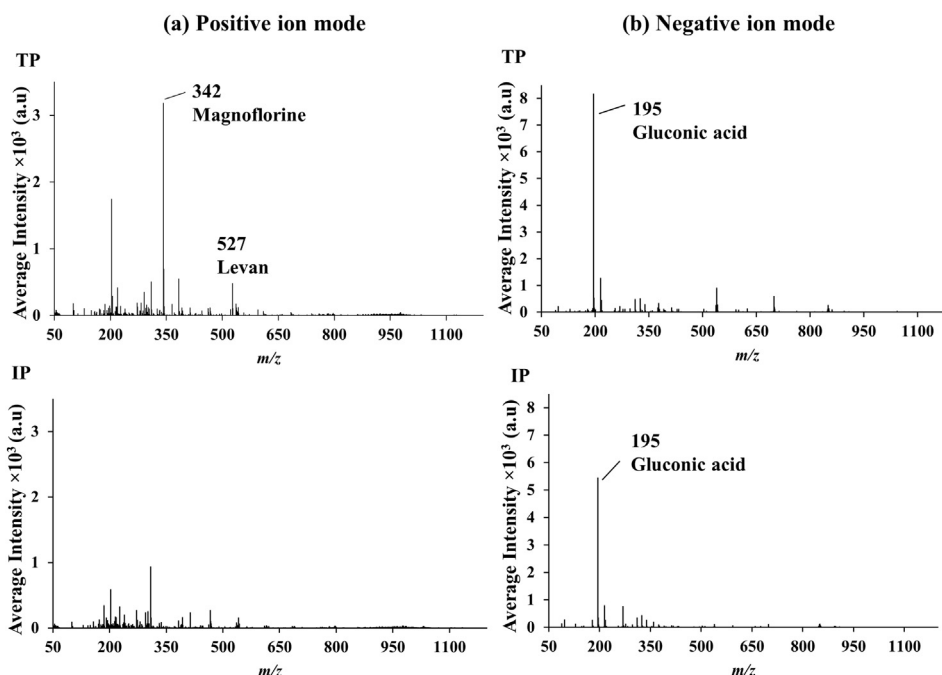


Fig. 2 – DESI-MS spectra of traditional Pru (TP) and industrial Pru (IP). (a) Positive and (b) negative ion mode spectra are shown in the left and right panel, respectively. In the positive ion mode, two ions at m/z 342 and m/z 527, proposed as magnoflorine and levan, respectively, were exclusively observed in traditional Pru. In the negative ion mode, the ion at m/z 195, proposed as gluconic acid, was the most abundant in both traditional and industrial Pru. a.u: arbitrary unit.

Table 2 – List of m/z values of precursor ions along with their MS/MS fragments obtained from TP and IP by FTICR MS/MS analyses (Molecular identities were not confirmed).

Precursor ion (m/z observed)	MS/MS fragments (observed)	Molecular assignments	TP	IP
Positive ion				
203.05	–	–	○	○
290.07	200.04, 201.05	–	○	×
380.11	–	–	○	○
383.11	203.05, 290.07	–	○	○
543.13	272.06	–	○	×
595.13	415.07	–	○	×
Negative ion				
179.09	–	–	○	○
191.05	–	–	○	×
215.03	179.06	–	○	○
219.05	–	–	○	×
269.08	179.05, 267.07, 68.06, 125.27	–	○	○
277.03	–	–	○	○
297.08	267.07	–	○	○
315.01	–	–	○	×
359.12	179.06, 161	–	○	○
375.11	195.05	–	○	○
391.11	195.05, 213.01, 341.1, 387.11, 359.12, 207.05	–	○	○
539.14	503.16	–	○	○
595.13	415.07	–	○	○

○: Observed; ×: Not observed.

4. Discussion

To our knowledge, this is the first study to analyze the major components of Pru, a Cuban traditional beverage produced both traditionally and industrially. Our highly sensitive and rapid analysis using DESI-MS and FTICR MS/MS revealed several compounds with promising beneficial properties. The observed MS/MS spectra of assigned compounds in our study are in excellent agreement with those published previously [17–21].

We found gluconic acid as the most abundant shared compound between TP and IP (Table 1). Gluconic acid has been extensively discussed in literature for its beneficial health effects, indicating it as one of the key compounds in Pru obtained by both traditional and industrial methods. Gluconic acid, which is widely distributed in nature (e.g., rice, honey, fruits, wine, vinegar, meat, dairy products, etc.) as well as produced by different microorganisms including bacteria, fungi, and yeasts, is commonly added to foods and drugs as an acidity regulator, and is also considered an excellent chelator of the anions of calcium, aluminum, iron, copper, and other heavy metals, especially in alkaline medium [22]. Gluconate and gluconolactone, the cyclic ester of gluconic acid, exist in chemical equilibrium and are reported to increase the plasma levels of glutathione, thereby exhibiting antioxidant activity [23]. These data indicate that some beneficial health effects of Pru could be attributed to gluconic acid.

The most striking finding of our study is the exclusively high abundance of magnoflorine in TP. Previously, this compound has been reported to decrease arterial blood pressure in rabbits [24] and to play a role in protecting

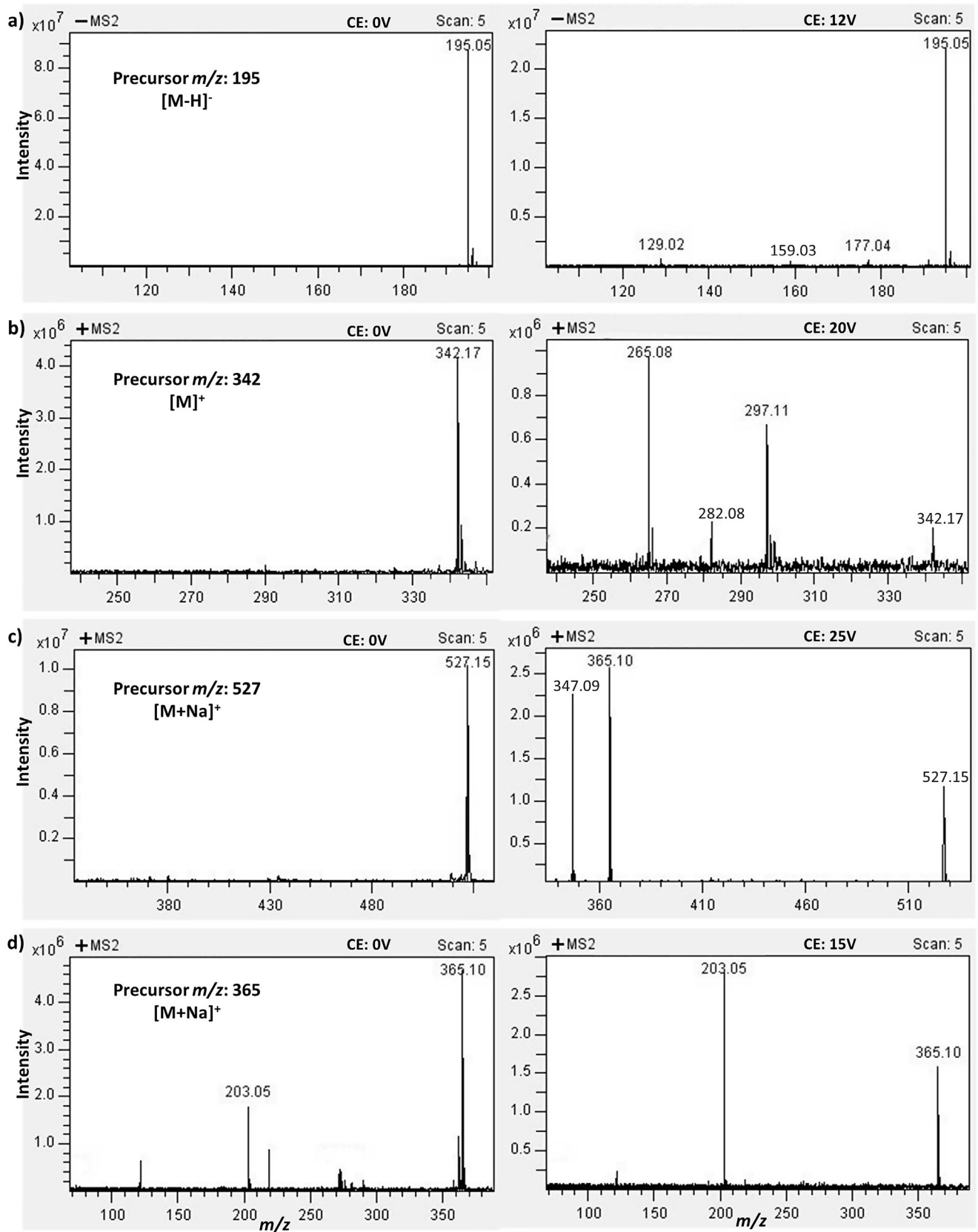


Fig. 3 – FTICR MS/MS spectra of a) gluconic acid, b) magnoflorine, c) levan, and d) sucrose. Gluconic acid and sucrose were detected in both TP and IP, whereas, magnoflorine and levan were exclusively detected in TP. CE: Collision Energy.

high-density lipoprotein (HDL) under oxidative stress [25]. Magnoflorine isolated from *Aristolochia debilis* has been reported to have significant antioxidant activity [26]. These data led us to assume that some beneficial effects, including the claimed hypotensive effect of Pru could be attributed to this compound.

Levan, an unusual polysaccharide, is another important molecule exclusively found in TP with high abundance. Levan is known to be produced by several dozen bacteria when grown on sucrose and is reported to lower blood cholesterol in animals [27] suggesting the health effects of TP.

Besides the ions for magnoflorine and levan, a number of non-identified ions (Table 2) were exclusively observed in TP suggesting a marked discrimination between TP and IP. This observed variation could be attributed to the method of production of this beverage, such as variation in the herbal composition and the duration of fermentation.

We also confirmed the presence of sucrose in both TP and IP. Since sucrose is an integral raw material of Pru production, detection of ions for this compound is expected.

DESI-MS has been employed in the current study as it requires no extraction and is capable of analyzing beverage samples rapidly. DESI-MS for crude sample analysis potentially avoid any contaminants from insoluble molecules as its sample flow is physically separated from a solvent flow. The capability of confirming the origin of each signal by constructing the ion images corresponding to the m/z values is an additional advantage of this technique. However, one major problem with DESI-MS is that liquid samples blow away and/or splash from the surface immediately by the high-velocity nebulizing nitrogen gas used for the production of ionized microdroplets. Consequently, the resulting ion signals from the sample do not last long and ion signals from the background dominate. Previously, metabolites and drugs from dried urine samples were successfully analyzed by employing this technique [14,15]. In this study, we successfully overcame this issue by allowing the sample to dry and then scanning across the entire droplet area instead of directly probing at a certain point on the droplet.

The study revealed the potential applications of Pru, preferably that prepared through traditional methods, in extending human health span. Therefore, the study is expected to promote the use of this beverage throughout other countries. Our study also suggested that DESI-MS could be a suitable tool for the rapid analysis of beverages. Further studies are needed to determine the correlation between the health benefits and the metabolite concentrations in this beverage.

5. Conclusion

The present study successfully revealed some major components of TP and IP, and discriminated between these two beverages. Gluconic acid was found to be the most abundant shared molecule between TP and IP, whereas magnoflorine and levan were exclusively detected in TP reflecting the better quality of Pru obtained by traditional methods.

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Conflicts of interest

We declare that we have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jfda.2019.05.004>.

REFERENCES

- [1] López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013;153:1194–217. <https://doi.org/10.1016/j.cell.2013.05.039>.
- [2] WHO | Facts about ageing. WHO 2015. www.who.int/ageing/about/facts/en/ [accessed October 2018].
- [3] Steinkraus KH. *Handbook of indigenous fermented foods*. Marcel Dekker; 1995.
- [4] Corrêa RCG, Peralta RM, Haminiuk CWI, Maciel GM, Bracht A, Ferreira ICFR, et al. New phytochemicals as potential human anti-aging compounds: reality, promise, and challenges. *Crit Rev Food Sc Nutr* 2018. <https://doi.org/10.1080/10408398.2016.1233860>.
- [5] Gomez-Zavaglia A, De Los Angeles Serradell M, Ozogul F, Tamang JP, Shin D-H, Jung S-J, et al. Functional properties of microorganisms in fermented foods. *Front Microbiol* 2016;7:578. <https://doi.org/10.3389/fmicb.2016.00578>.
- [6] Eisenberg T, Abdellatif M, Schroeder S, Primessnig U, Stekovic S, Pendl T, et al. Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat Med* 2016;22:1428–38. <https://doi.org/10.1038/nm.4222>.
- [7] Pellegrino D. Antioxidants and cardiovascular risk factors. *Diseases (Basel, Switzerland)* 2016;4. <https://doi.org/10.3390/diseases4010011>.
- [8] González-Burgos E, Liaudanskas M, Viškelis J, Žvikas V, Janulis V, Gómez-Serranillos MP. Antioxidant activity, neuroprotective properties and bioactive constituents analysis of varying polarity extracts from *Eucalyptus globulus* leaves. *J Food Drug Anal* 2018;26:1293–302. <https://doi.org/10.1016/j.jfda.2018.05.010>.
- [9] Volpato G, Godínez D. Ethnobotany of Pru, a traditional Cuban refreshment. *Econ Bot n.d.*;58:381–395. <https://doi.org/10.2307/4256833>.

- [10] Zaima N, Hayasaka T, Goto-Inoue N, Setou M. Matrix-assisted laser desorption/ionization imaging mass spectrometry. *Int J Mol Sci* 2010;11:5040–55. <https://doi.org/10.3390/ijms11125040>.
- [11] Ide Y, Waki M, Hayasaka T, Nishio T, Morita Y, Tanaka H, et al. Human breast cancer tissues contain abundant phosphatidylcholine(36:1) with high stearyl-CoA desaturase-1 expression. *PLoS One* 2013;8:e61204. <https://doi.org/10.1371/journal.pone.0061204>.
- [12] Hosokawa Y, Masaki N, Takei S, Horikawa M, Matsushita S, Sugiyama E, et al. Recurrent triple-negative breast cancer (TNBC) tissues contain a higher amount of phosphatidylcholine (32:1) than non-recurrent TNBC tissues. *PLoS One* 2017;12:e0183724. <https://doi.org/10.1371/journal.pone.0183724>.
- [13] Hossen MA, Nagata Y, Waki M, Ide Y, Takei S, Fukano H, et al. Decreased level of phosphatidylcholine (16:0/20:4) in multiple myeloma cells compared to plasma cells: a single-cell MALDI–IMS approach. *Anal Bioanal Chem* 2015;407:5273–80. <https://doi.org/10.1007/s00216-015-8741-z>.
- [14] Parrot D, Papazian S, Foil D, Tasdemir D. Imaging the unimaginable: desorption electrospray ionization – imaging mass spectrometry (DESI-IMS) in natural product research. *Planta Med* 2018;84:584–93. <https://doi.org/10.1055/s-0044-100188>.
- [15] Kauppila TJ, Talaty N, Kuuranne T, Kotiaho T, Kostianen R, Cooks RG. Rapid analysis of metabolites and drugs of abuse from urine samples by desorption electrospray ionization-mass spectrometry. *Analyst* 2007;132:868. <https://doi.org/10.1039/b703524a>.
- [16] Nakabayashi R, Sawada Y, Yamada Y, Suzuki M, Hirai MY, Sakurai T, et al. Combination of liquid chromatography–Fourier transform ion cyclotron resonance-mass spectrometry with ¹³C-labeling for chemical assignment of sulfur-containing metabolites in onion bulbs. *Anal Chem* 2013;85:1310–5. <https://doi.org/10.1021/ac302733c>.
- [17] Felipe D, Brambilla L, Porto C, Pilau E, Cortez D, Felipe DF, et al. Phytochemical analysis of *Pfaffia glomerata* inflorescences by LC-ESI-MS/MS. *Molecules* 2014;19:15720–34. <https://doi.org/10.3390/molecules191015720>.
- [18] Singh A, Bajpai V, Srivastava M, Arya KR, Kumar B. Rapid profiling and structural characterization of bioactive compounds and their distribution in different parts of *Berberis petiolaris* Wall. ex G. Don applying hyphenated mass spectrometric techniques. *Rapid Commun Mass Spectrom* 2014;28:2089–100. <https://doi.org/10.1002/rcm.7001>.
- [19] Singh A, Bajpai V, Kumar S, Singh Rawat AK, Kumar B. Analysis of isoquinoline alkaloids from *Mahonia leschenaultia* and *Mahonia napaulensis* roots using UHPLC-Orbitrap-MSn and UHPLC-QqQLIT-MS/MS. *J Pharm Anal* 2017;7:77–86. <https://doi.org/10.1016/j.jpfa.2016.10.002>.
- [20] Metlin. <https://metlin.scripps.edu/showChart.php?molid=6945&h=240&collE=20&Imode=p&etype=experimental> [accessed March 2019].
- [21] Metlin. <https://metlin.scripps.edu/showChart.php?molid=137&h=240&collE=20&Imode=p&etype=experimental> [accessed March 2019].
- [22] Ramachandran S, Fontanille P, Pandey A, Larroche C. Gluconic acid: properties, applications and microbial production. *Food Technol Biotechnol* 2006;44:185–95.
- [23] Kolodziejczyk J, Saluk-Juszczak J, Wachowicz B. In vitro study of the antioxidative properties of the glucose derivatives against oxidation of plasma components. *J Physiol Biochem* 2011;67:175–83. <https://doi.org/10.1007/s13105-010-0061-3>.
- [24] El Tahir KEH. Pharmacological actions of magnoflorine and aristolochic acid-1 isolated from the seeds of *Aristolochia bracteata*. *Int J Pharmacogn* 1991;29:101–10. <https://doi.org/10.3109/13880209109082858>.
- [25] Hung TM, Lee JP, Min BS, Choi JS, Na M, Zhang X, et al. Magnoflorine from *Coptidis Rhizoma* protects high density lipoprotein during oxidant stress. *Biol Pharm Bull* 2007;30:1157–60.
- [26] Li C, Wang M-H. Potential biological activities of magnoflorine: a compound from *Aristolochia debilis* Sieb. et Zucc. *Korean J Plant Resour* 2014;27:223–8. <https://doi.org/10.7732/kjpr.2014.27.3.223>.
- [27] Combie J. Properties of levan and potential medical uses. 2006. p. 263–9.