Effect of spray drying on the polyphenolic compounds present in purple sweet potato roots: Identification of new cinnamoylquinic acids.

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Abstract

The effect of spray drying on the different polyphenolic compounds present in the root of a purple-fleshed sweet potato variety of *Ipomoea batatas* native from Peru was performed by HPLC-QTOF-MSMS. Nine anthocyanins, including four peonidin, three cyanidin and two pelargonidin derivatives glycosylated with sophorose and/or glucose and acylated with caffeic, ferulic and *p*-hydroxybenzoic acid were identified. Twenty nine cinnamoylquinic acids (CiQA), including eight mono-CiQA, fourteen di-CiQA, and five tri-CiQA, were identified on the base of their MS fragmentation profile. Relevant amounts of feruloylquinic acid derivatives were identified. Among them, some di and tri-CiQAs containing feruloyl and caffeoyl moieties in their structures, and di-feruloylquinic acids were reported here, for the first time, in *Ipomoea* . Spray drying process negatively affected the different phenolic groups, with polyphenol losses representing around 90% of the initial amounts. Mono-CiQAs presenting feruloyl moieties and mono acylated peonidin derivatives with p-hydroxybenzoic acid were the less affected compounds.

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Key words; purple sweet potato, *Ipomoea batatas*, anthocyanin, cinnamoyl-quinic acid, spray-drying, HPLC-QTOF-MSMS, chlorogenic acid, feruloylquinic acid derivatives

Highlights

- Purple sweet-potato contains relevant amounts of feruloylquinic acids derivatives
- Identification of new di and tri cinnamoylquinic acids in Ipomoea batatas roots
- The spray drying process reduced cinnamoylquinic acids, anthocyanins and flavonols
- Mono cinnamoylquinic acids containing feruloyl moieties were less degraded
- Peonidin acylated with *p*-hydroxybenzoic acid was the most thermostable anthocyanin

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Introduction

Sweet potato (*Ipomoea batatas* L.), a dicotyledonous plant of the family *Convolvulaceae*, is a rich source of carbohydrates and, in a lower extend, of other nutrients such as proteins and lipids. Sweet potato roots and other aerial parts of the plant are used as human food and animal feed but also for the development of industrial products (eg starch, sugar and natural colorants). It also contains functional components (as polyphenols) that have been used in folk medicine for the treatment of a wide variety of pathological conditions (Meira, Pereira da Silva, David, & David, 2012). In the last years, the development of dietary foods, snacks and vegetable juices including sweet potatoes has been increased (Truong, Avula, Pecota & Yencho, 2018). Industrial processing usually involves making the roots into purees that can be subsequently preserved by dehydration processes that might affect the characteristics and quality of sweet potato flours

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Among the drying techniques, spray drying is one of the most rapid and less expensive procedures. During the drying process the material is sprayed into a chamber where hot dry air rapidly evaporates the small droplets leaving the spray dried particles. Spray-drying has been proposed for commercial production of functional ingredients from several fruits and vegetables including sweet potatoes (Grabowski, Truong & Dauber, 2008). However, the high temperatures used during the process might affect the polyphenol content and antioxidant activity of the final product. In this regard, the degradation of polyphenols of *Ipomoea batatas* under different cooking conditions has been reported (Takenaka, Nanayama, Isobe & Murata, 2006; Kim et al., 2012; Xu et al., 2015). Still, the impact of spray drying on the different polyphenolic compounds present in *Ipomoea batatas* has not been reported so far.

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On the other hand, freeze drying is a process in which the water is removed from a frozen solution by under reduced pressure not requiring an increase in the temperature and thus, preserving most of the initial raw material properties. However, freeze drying is time-consuming and less used in food industry due to its high operation cost (Ratti, 2001)

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Polyphenolic compounds in sweet potatoes are mainly concentrated in leaves followed by peel and the whole root. Among them, chlorogenic acid derivatives (mainly mono and di caffeoylquinic acid derivatives, CQAs) and anthocyanins (especially abundant in purple-fleshed sweet potatoes varieties) are the most abundant polyphenolic groups identified in *Ipomoea batatas* roots of varieties from different origins (Ishiguro, Yahara & Yoshimoto, 2007; Truong, Mcfeeters, Thompson, Dean & Shonfran, 2007; Truong et al. 2010).

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Chlorogenic acids are a family of esters, widely distributed in plants, formed between quinic acid and certain *trans*-cinnamic acids, most commonly caffeic, *p*-coumaric and ferulic acid. The most common chlorogenic acids reported in sweet potatoes are 3-O-caffeoylquinic acid (3CQA), 4-O-caffeoylquinic acid (4CQA) and 5-O-caffeoylquinic acid, and some dicaffeoylquinic acids such as 3,4-diO-caffeoylquinic (3,4-diCQA), 3,5-di-O-caffeoylquinic acid (3,5-diCQA) and 5-di-O-dicaffeoylquinic acid (4,5-diCQA). These compounds are present in all parts of plants, and factors such as plant physiological state, storage conditions but also temperature during drying process might affect its qualitative and quantitative composition (Ishiguro et al. 2007; Terahara et al. 1999).

Purple sweet potatoes exhibit an intense purple colour due to the accumulation of mono- and diacylated anthocyanins, mainly cyanidin and peonidin derivatives glycosylated with sophorose and/or glucose. These compounds are acylated with caffeoylquinic acid, *p*-hydroxybenzoic acid or ferulic acid (Terahara et al. 1999). The rate of acylation confers stability to the anthocyanins (Hayashi, Ohara & Tsukui, 1996; Xu et al. 2015) and purple-fleshed sweet potato is considered a valuable plant material for the production of natural food colorants (Suda, Oki, Masuda, Kobayashi, Nishiba & Furuta, 2003). Comparatively, anthocyanins extracted from purple-fleshed sweet potato exhibits higher colour stability than those extracted from plants such as raspberry, red cabbage, strawberry or apple, owing to their unique mono- or di- acylated forms (Hayashi et al 1996; Kim et al.2012). In addition to anthocyanins, the presence of cinnamoylquinic acids in these varieties contributes to their antioxidant activity as well as to the phytochemical and colour stability of the anthocyanins present in Ipomoea (de Aguilar Cipriano, Ekici, Barnes, Gomes, & Talcott, 2015). However, studies dealing

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with the characterization of both groups of polyphenols simultaneously in *Ipomoea batatas* are limited (Zhao et al. 2014). Despite the potential of spray drying in converting sweet potatoes into functional ingredients, the impact of such technology on the different bioactive compounds present in *Ipomoea batatas* roots remains unknown. Thus, the objective of this study was to study the effect of spray drying on the different polyphenol groups, including cinnamoylquinic acids, anthocyanins and flavonols, present in the root of a purple-fleshed sweet potato variety of *Ipomoea batatas* native from Peru.

2. Materials and methods

2.1. Chemicals

All chemical used were HPLC analytical grade. Caffeic acid, 5-*O*-caffeoylquinic acid, *p*-coumaric acid, cyanidin 3-*O*- glucoside, quercetin 3-*O*-glucoside, L-phenylalanine and L-tryptophan were purchased from Sigma Chemical Co (St. Louis, MO).

2.2. Sample preparation and dehydration processes

Purple sweet potato roots of *Ipomoea batatas L Lam* variety and commonly so-called “Camote morado de Piura” were grown at Piura Catacaos (Piura, Peru). After 5 months, plants were harvested and roots were separated, washed with tap water and then were subjected to freeze dried or spray dried process. Freeze drying process was performed in a Stellar ® Laboratory Freeze Dryer (Millrock Technology, Inc). After washing, whole roots were sliced (2 mm), placed in a tray, freezed (-20 ºC) and introduced in the Freeze Dryer during 12 hours, with a temperature starting from −40 °C and progressing towards 10 °C. Dried roots were crushed to powder with the help of a mortar and stored in an aluminium bag until their analysis. The spray-drying was conducted using an A/S Niro NF Atomizer® (Danemark). Before being introduced in the atomizer, whole washed roots were mashed by adding water (50:50) in a food processor (Jefc60, Imeco, Peru) at room temperature. A carrier (10% maltodextrine , Maltogill 20, Dextrose Equivalent-DE 20) was added to the puree and

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introduced in the atomizer. The flux was 1 litre per hour and the inlet and outlet temperatures were 160-180 ºC and 85-95 º C, respectively. Dried powdered roots were stored in an aluminium bag until analysis.

2.3. Polyphenol extraction

For the extraction of phenolic compounds, 50 mg of sample was placed in an Eppendorf, suspended in 1 ml of MeOH/H2O (50:50 v/v acidified with formic acid 0.1%), vortexed and sonicated during 15 minutes. Samples were then centrifuged (Eppendorf AG, Hamburg, Germany) at 10.000 rpm for 15 min at 4°C and the supernant was collected. The residue was re-suspended in 0.5 ml of MeOH/H2O (1:1 acidified with 0.1% formic acid) and re-extracted following the same procedure two times. Supernants were combined, filtered (0.45 µm), and placed in vials for subsequent HPLC-QTOF-MSMS analysis of phenolic Compounds.

2.4. HPLC-QTOF-MS Analysis of Phenolic Compounds.

Analyses were performed using HPLC coupled with a mass spectrometer (HPLC-MS- QTOF). The HPLC (Agilent 1200, Agilent Technologies) with a quaternary pump (G1311A) was coupled with diode array detector (Agilent G1315B) and an Agilent 6530 Accurate-Mass QTOF LC /MS with Electrospray Ionization (ESI) with Jet Stream technology (Agilent Technologies). Separation was performed on a Phenomenex Luna C18 column (5 µm, 4.6 mm × 150 mm), set thermostatically at 25 °C.

A gradient between solvent A (water/formic acid, 99.9:0.1, v/v) and solvent B (acetonitrile/formic acid, 99.9:0.1, v/v) was applied at a flow rate of 0.5 mL/min as follows: 10% B at 0 min, 30% B at 30 min, 35% B at 35 min, 40% B at 45 min, 10% B at 50 min, and 10% at 60 min. The volume of sample injected was 20 µL. The electrospray ionization (ESI) parameters were as follows: drying gas flow, 8 L/min; nebulizer pressure, 45 psi; gas drying temperature, 325 °C; sheath gas temperature, 300 °C; sheath gas flow, 11 L/min; capillary voltage, 4000 kV; and fragmentator, 120 V. The ESI was operated in positive and negative modes to provide extra certainty in the determination of the molecular masses. Data were collected in extended dynamic range, 100−1200 m/z. For the identification and quantification of compounds MS and tandem mass spectrometry fragmentation spectra (MSMS) experiments were performed and spectral signal at data were also acquired at 280 and 320 and 520 nm. For MSMS experiments, quite generic collision energy of 20 V was used, as a compromise, to simplify development of the method and ensure good fragmentation of the majority of targeted compounds. Data acquisition and processing were performed with the Masshunter Data Acquisition B.05.01 and Masshunter Qualitative Analysis B.07.00 SP2 software. Compounds were identified by comparing mass spectra and retention time with the corresponding standard if available. In the case of compounds for which standards were not available, identification was based on prediction of chemical formula from accurate ion mass measurement and confirmed by comparing MSMS with data provided by relevant literature references (see tables 1 and 3). The quantification was performed by interpolation into the calibration curve of the standard or structurally related compound used to quantify (equivalent) and expressed as µg per g of dry matter (DM) as follow: 5-*O*-caffeoylquinic acid for hydroxycinnamic acid derivatives, cyanidin-3-O- glucoside for anthocyanins, and quercetin-3-*O*-glucoside for flavonols.

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2.5. Statistical analysis

All analyses were performed in quadruplicate. Data were subjected to a one-way analysis of variance (ANOVA) by using the general linear model procedure (Version 9.2, SAS Inst. Inc., Cary, NC). Variance homogeneity was performed using Levene's test. When the effect was considered significant ($P<0.05$), means were compared using the Tukey's range test.

3. Results and discussion

3.1 Cinnamoyl-quinic acids

Cinnamoylquinic acids (CiQAs), also known as chlorogenic acids, are esters formed between certain *trans*-cinnamic acids (caffeic, ferulic and *p*-coumaric) and quinic acid. These compounds are characteristic of coffee beans, and LC-MSn fragmentation behaviour of their main components has been widely described by Clifford and Kuhnert using mild fragmentation conditions (Clifford, Johnston, Knight & Kuhnert, 2003; Clifford, Knight & Kuhnert, 2005; Clifford, Knight, Surucu & Kuhnert, 2006a; Clifford, Marks, Knight & Kuhnert, 2006b; Clifford, Wu, Kirkpatrick, & Kuhnert, 2007; Jaiswal & Kuhnert 2010) and it has been used to confirm the identity of the CiQAs in this paper. These authors established hierarchical keys for CiQAs derivatives identification based on MSn fragmentation patterns, relative hydrophobicity and bonding strength to quinic acid. We have used the numbering nomenclature recommended by the International Union of Pure and Applied Chemistry (IUPAC 1976) for quinic acid derivatives, and in order to clarify, previous data published using other systems have been transformed to IUPAC nomenclature. Characterization of the CiQAs was performed in negative mode and is reported in table 1 and figure 1.

In the present study, free caffeic acid (CA) with a molecular ion at m/z 179 was identified by comparison with the corresponding commercial standard and on the basis of the MSMS fragmentation pattern m/z 135 originated from the decarboxylation of caffeic acid.

3.1.1 Mono- Cinnamoyl-quinic acids

Among the mono cinnamoyl-quinic acids, the most abundant single compounds identified in freeze-dried purple sweet potato were those including cafffeic and ferulic acid in which the esterification occurs at position 5, corresponding to 5 caffeoylquinic acid (5CQA) and 5 feruloylquinic acid (5FQA) with m/z 353 and 367, respectively. Although the esterification might occurs at positions 1, 3, 4, and 5 of the quinic acid moiety, only the isomers with esterification at positions 3, 4, and 5 have been previously reported in *Ipomoea batatas*. The distinctive behaviour of the different isomers during the MS fragmentation along with the use of a reference standard (5CQA) allowed distinguishing them.

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In this sense, it was observed that the CQA with a caffeoyl residue at C5 was the most easily fragmented (yielding a fragment at m/z 191), whereas that at C4 was the most stable (yielding m/z 173 and 135) and that at C3 being of intermediate stability (yielding at 191 and 135). Additionally, a peak at 15.5 minutes showed the same fragmentation pattern than 5CQA and was assigned to the *cis*-form of 5CQA. Although it is widely accepted that the hydroxycinnamic acid moiety of acyl-quinic acid is predominantly found in the *trans* form, UV-irradiation may cause geometric isomerization and *cis* derivatives have been detected in other plant species (Clifford, Wu, Kirkpatrick, Kuhnert, Roozendaal & Rodrigues Salgado, 2008). In accordance to these authors, *cis* and *trans* isomers get fragmented identically and their order of elution depends on the position of substitution, being 5CQA more hydrophobic and thus eluting later than their mono- or di-*trans* counterparts whereas the reverse is true for 3 and 4 *cis* CQA.

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Three peaks presenting a common molecular ion at m/z 367 were assigned as FQAs. The main ions produced for each successive peak during fragmentation were m/z 193, 173 and 191, respectively which, according to the hierarchical keys proposed by Clifford et al. 2003, allowed their identification as 3FQA, 4FQA and 5FQAs, respectively. However, and taking into account that according to these authors FQA isomers elute in the same order as CQAs (3, 5, and 4), the last compound was tentatively assigned to the cis-isomer. Despite the presence of a cis-isomer is expected to be accompanied, and indeed dominated by the corresponding trans-isomer, we were not able to find the trans-isomer of 5FQA. In this sense, the predominance of 4FQA was surprising as the esterification at position 5 is most common in the plants presenting these compounds. The presence of FQAs isomers have been rarely described in *Ipomoea batatas* where only few amount of 3FQA isomer has been reported (Zhu, Cai, Yang, Jinxia & Corke, 2010; Wang et al. 2018) in roots.

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A peak with molecular ion at m/z 337 was identified as *p*-coumaroylquinic acid (pCoQA). Attending to the presence of a fragment at m/z 191 it was assigned as 5pCoQA. The presence CoQAs has been also reported in roots of chinese varieties of sweet potato (Wang et al. 2018).

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In the present variety the group of CQA derivatives (with 5CQA as the most abundant compound) was the most representative group (53%) followed by FQAs (42%) and pCoQAs (4,5%). According to previous reports (Takenaka et al. 2006; Jung 2011) the content of CQAs in *Ipomoea batatas* varies greatly with the variety, being 5CQA the most abundant compound found in roots (ranging from 10 to 300 µg/g DM). In the present work 5CQA was also the predominant isomer and its content (68 µg /g) was within the range of previously reported values. Our results also showed that the root of this variety of purple sweet potato native of Peru possess an important content of FQAs where the presence of the 3 different isomers of FQA were identified. Cinnamoylquinic acids play an important role in the plant defence response, and the presence of FQAs, have been associated to additional protective resistance against plant pathogens (Nuringtyas, Choi, Verpoorte, Klinkhamer & Leiss, 2012).

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3.1.2. Di-cinnamoyl quinic acids

A peak with a molecular ion at m/z 499 corresponding to a *p*-coumaroyl-caffeoylquinic acid (pCoCQA) with a MS fragmentation pattern characterized by ions at m/z 353 and 191 was detected. Clifford et al. 2006b reported on the basis of the LC-MS^4 patterns of fragmentation the presence of 6 different isomers of pCoCQAs. Among them, three yielded a common base MS peak at m/z 353, but only one (corresponding to 3C5pCoQA) was also accompanied with a secondary peak at m/z 191 and consequently was assigned to this compound. To our knowledge, this compound has not been identified either in the roots or in the leaves of *Ipomoea batatas* previously.

Three di-caffeoylquinic acids (diCQA) with the expected m/z 515 were detected. According to the fragmentation pattern and elution order (Clifford et al. 2003 and 2005) two of them producing the characteristic peak at m/z 173 that indicated a substitution at position 4, were assigned to 3,4diCQA and 4,5diCQA. Another isomer producing a fragment at m/z 191 was associated to 3,5diCQA. Accordingly, these isomers have been commonly reported in *Ipomoea batatas* roots (Truong et al. 2007; Jung, Lee, Kozukue, Levin & Friean, 2011; Park, Woo, Choi, Choi, & Jung, 2015).

Seven peaks showing molecular ions at m/z 529 were identified as isomers of caffeoyl-*O*-feruloylquinic acid (CFQAs). Zheng and Clifford (2008) reported the presence of low concentration of 6 isomers of CFQA in the stem of *Ipomoea batatas*. Different CFQA isomers have been widely reported in robusta coffee beans (Clifford et al. 2003 and 2006a and b). The CFQAs were tentatively assigned according their MSn fragmentation pattern and elution order (Clifford et al. 2006a). The MSMS base peak at *m*/*z* 353 or 367, along with the presence of the characteristic secondary ions (as *m*/*z* 173 indicating position 4) and the sequence of elution allowed the assignment of the different isomers. In this sense the absence of the secondary ion at *m*/*z* 173 indicated the presence of 3,5-diacyl derivatives of hydroxycinnamic acid, whereas its presence indicated 3,4 or 4,5-diacyl derivatives. Regarding the 3,5-diacyl derivatives, 3 peaks (31.6, 34.2 and 34.6) were observed, two of them presenting a similar MS fragmentation pattern (m/z 367 and 193) and suggested the presence of 2 isomers (*cis* and *trans*) of 3F5CQA, whereas another peak produced m/z 353 and 191 was assigned to 3C5FQA. For the 3,4 or 4,5-diacyl derivatives, because the fragmentation pattern do not allow to assign the position of the substituents of both hydroxycinnamic acids (caffeic and ferulic acid), the identification was performed according to the same elution order followed for diCQAs and reported by Clifford et al. 2006a .

Three peaks yielding molecular ion signals at m/z 543 and presenting and identical fragmentation pattern and elution order as diFQA (Clifford et al. 2006a) were identified. Accordingly, these peaks with the fragmentation pattern of m/z 349/173, m/z 367/193 and m/z 367/173 were respectively assigned to 3,4diFQA; 3,5diFQA and 4,5diFQA. To our knowledge, these compounds have not been identified previously either in the roots or in the leaves of *Ipomoea batatas*.

One peak with m/z 677 with a main fragmentation pattern m/z 515, 353, 173 suggesting the presence of 3,4,5 tri-*O*-caffeoylquinic acid (3,4,5tCQA) according to MS^n information provided by Clifford et al. (2007) and Jaiswal and Kuhnert (2010) in Chrysanthemum and coffee bean was observed. The presence of this compound in leaves of *Ipomoea* has been reported (Islam et al. 2002; Luo et al., 2013). However, their earlier elution together with the additional presence of, although weak, MSMS

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fragments at m/z 497 and 341, also suggested the presence of a dicaffeoylquinic acid glycoside as was described in Chrysanthemum (Clifford et al. 2007), but not in *Ipomoea batatas*. In this sense, the loss of a fragment with m/z 162 may correspond to the loss of a caffeic acid or a hexose, being the latter more hydrophilic and therefore eluting earlier.

As was observed for mono CiQAs, a relevant content of di CiQAs containing feruloyl moieties was identified in this variety. Despite, as expected, dicaffeoylquinic acids were the most abundant group of total diCiQAs, a valuable 42% of total diCiQAs corresponded to compounds presenting both feruloyl and caffeoyl quinic acids which were reported here for the first time in *Ipomoea batata*s roots. Additionally, low amounts of total diCiQAs corresponded to diferuloylquinic acids and pCoCQAs (2,7 and 0.7 %, respectively) were not previously reported in sweet potatoes.

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3.1.3. Tri cinnamoyl quinic acids

Two peaks yielding molecular ion signal at m/z 691 were identified as feruloyl-*O*-dicaffeoylquinic acid (FdCQAs) according to the molecular mass, pattern of fragmentation and elution order previously described in green robusta coffee beans (Jaiswal and Kuhnert 2010). These authors identified and characterized for the first time three structures containing feruloyl (position at 3, 4 and 5) and dicaffeoylquinic acids (positions at 3,4, 3,5 and 4,5). Accordingly, the fragmentation of the first two eluting compounds produced a common peak at m/z 529 followed by m/z 367,173 or 353,173, 179 and were assigned to 3,5di-*O*-caffeoyl-4-*O*-feruloylquinic acid and 3-*O*-feruloyl-4,5-di-*O*-caffeoylquinic acid (3,5diC4FQA and 3F4,5CQA, respectively). These authors also reported a third compound assigned as 3,4-*O*-dicaffeoyl-5-*O*-feruloylquinic acid (3,4diC5FQA) which produced characteristics MSMS base peak at m/z 515 (diCQA), MS^3 base peak at m/z 353 (supported by a secondary m/z 255 and 299), and MS4 based peaks at 173 and 179.

The MS^2 fragmentation pattern observed in the present study showed that the first eluting compound produced ions at m/z 515, 299 and 255 also showed intermediate weak peaks at 529, 353 and 173 and was partially similar to that reported as 3,4diC5FQA. Regarding our second eluting compound, the

MSMS spectra (m/z 529/353/179) produced was similar to that reported for 3F4,5CQA. However, and due to the discrepancies with the elution order previously proposed for coffee beans, we couldn’t confirm the identity of such isomers and, consequently, were assigned as dicaffeoyl-feruloylquinic acid 1 and 2, respectively. To our knowledge, these compounds have been scarcely reported in nature, and are reported here for the first time in *Ipomoea batatas*.

Three peaks eluting between 40.5 and 42.0 min yielded molecular ion signal at m/z 705 were assigned to caffeoyl-*O*-diferuloylquinic acid derivatives. Compounds were identified by comparing their MSMS fragmentation spectra with those previously reported by Jaiswal and Kuhnert (2010). Our first eluting compound with MSMS spectra of m/z 529, 367 and 173 was similar to that reported for 4,5-*O*-diferuloyl-3-*O*-caffeoylquinic acid. The second one, producing a MSMS spectra with m/z 543/367/349 /193 was also similar to that assigned to 3,4-di-*O*-feruloyl-5-*O*-caffeoylquinic acid. Finally, we also observed a third eluting peak presenting a MSMS spectra with m/z 529/353/179 which was not previously reported in coffee bean and was named diferuloyl-O-caffeoylquinic acid 3.

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Around 10% of total CiQAs observed in purple sweet potato roots corresponded to tri CiQAs, where diCFQAs and CdiFQA (representing 90% and 6 % of the total tCiQAs, respectively) were the most abundant structures. In *Ipomoea batatas* mono and di acylated chlorogenic acids have been widely reported in all the parts of the plant whereas the presence of tri -acylated chlorogenic acids has been less documented. In this regard, the only structure previously reported corresponds to 3,4,5tCQA whose presence has been identified in the ipomoea leaves (Islam et al. 2002 ; Luo et al., 2013) and was not identified in the present study. However, we detail here for the first time in *Ipomoea batatas* the presence of feruloyl moieties in the structures of tri-acyl quinic acids. Full assignment of these compounds, and especially for structures presenting two different acyl moieties, requires MS^4 and even MS^5 spectra (Clifford, Jaganath, Ludwig & Crozier, 2017) and, therefore, only tentative identifications of such isomers are reported here.

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3.1.4. Effect of spray drying on Cinnnamoyl-quinic acids

The effect of spray drying on the CiQAs present in purple sweet potato roots is showed in table 2.

In *Ipomoea batatas*, mono and diCQAs are the most abundant phenolic acids present in roots from different varieties (Hayase & Kato 1984; Islam, Yoshimoto, Yahara, Okuno, Ishiguro & Yamakawa, 2002; Takenaka et al. 2006; Truong et al. 2006). In the present experiment, mono and di-acyl-quinic acids were also the most abundant groups of CiQAs in purple sweet potato, (each one representing around 45% of total CiQAs), followed by tri acyl-quinic acids (10 %).

The spray dried process resulted in CiQAs losses of around 87%. The reduction was less pronounced for mono CiQAs (72.7 %) than for diCiQAs and triCiQAs which were almost completely destroyed. The presence of a feruloyl moiety in the structure of CiQAs seemed to confer more thermal stability. In this sense, the losses observed after spray drying process were lower in mono FQAs than in mono CQAs (40.4 and 96.9 % reduction, respectively). CiQAs are highly susceptible to the influence of temperature. Temperature caused significant losses in these phenolic compounds and the severity was dependent on factors such as cooking (boiling, steaming, baking) and storage conditions of the roots (Takenaka et al. 2006; Jung et al. 2011). In these studies, a low stability of mono and di CQAs present in *Ipomoea batatas* was reported, however, the severity of the losses was dependent on the cooking techniques.

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Previous works suggest that spray drying is a satisfactory technique for encapsulation and drying of heat sensitive food ingredients including polyphenols (Fang & Bandhari, 2010). However, only few studies have reported on the effect of spray drying process on bioactive compounds present in sweet potato. In this sense, a reduction of nutrients including vitamin C and beta carotene was reported during the spray drying of orange sweet potato puree (Grabowsky et al. 2008). Regarding purple varieties, Peng, Li, Guan, & Zhao, (2013) reported a degradation of total polyphenols during the spray drying of sweet potato flours using spectrophotometric techniques, but the effect of such technology on the different cinnamoylquinic acids present in Ipomoea has not been studied. In these studies the rate of degradation ranged from 15 to 50% according to the different carrier and conditions used during the process. Correct selection of excipients during spray drying process is also an important

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step in guaranteeing the stability and quality of the final product. Among them, in the present study we used maltodextrin, which was reported to preserve better than other *Ipomoea batatas* flour polyphenols (Peng, Li, Guan, & Zhao, 2013). However, the conditions used in this experiment, were detrimental for the stability of the different individual phenolic compounds present in the roots of *Ipomoea batatas*. In addition to temperature, the process to which the roots were subjected before being spray dried might have contributed to the higher degradation of polyphenolic compounds observed in our case. In this sense, the grinding and homogenization of roots before spray drying led polyphenol present in purees more exposed to light and oxidative processes. The role of enzymes such us the polyphenol oxidase, released during plant damage, in the oxidation of CiQAs of Ipomoea might also contribute to the losses observed during processing (Takenaka et al. 2006). To inhibit the enzymatic activity and preserve the colour, some pre-treatments including heat and /or other inhibitors have been proposed and used during the industrial process of sweet potatoes (Truong et al. 2017) but were not included in the present experiment.

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3.2. Anthocyanins

Purple sweet potato (*Ipomoea batatas*) is a valuable plant material for the production of colouring foodstuffs. Anthocyanins extracted from purple-fleshed sweet potato present high thermostability owing to their unique mono- or di- acylated forms (Suda et al., 2003). Cyanidin and peonidin-3-sophoroside-5-glucoside acylated with *p*-hydroxybenzoic, ferulic, p-coumaric and caffeic acids are the main anthocyanins reported in purple-fleshed sweet potato (Terahara et al. 1999; Truong et al. 2010)

The chemical structures of *Ipomoea batatas* anthocyanins were determined in positive mode, and confirmed by comparing the MS fragmentation pattern with data previously reported (Tian, Konczak & Schwartz, 2005; Truong et al 2010). Considering that for most of the anthocyanins MS cleavage just occur between the anthocyanin and the glycoside, the presence of the aglycones with m/z 287, 301 and 271 allowed to identify cyanidin, peonidin and pelargonidin derivatives, respectively. The chemical structures of the individual anthocyanins were then determined considering in the fragmentation pattern the loss of the sugars, such as glucose (m/z 162) and sophorose (m/z 324), and the acylation groups such as p-hydroxy-benzoic acid (m/z 120), caffeic acid (m/z 162) and ferulic acid (m/z 176). Characterization of the anthocyanins is reported in table 3 and figure 2.

3.2.1. Cyanidin derivatives

Cyanidin derivatives were identified based in the presence of their characteristic aglycon fragment at m/z 287. Among them, the first molecular ion of m/z 773 was identified as cyanidin 3-sophoroside-5-glucoside according to its fragmentation pattern (see figure 2A). In this sense, the ion fragments at m/z 611 corresponded to the loss of a glucose (162), whereas m/z 449 corresponded to a loss of a sophorose (324). Another two cyanidin derivatives acylated with *p*-hydroxybenzoic acid and ferulic acid were identified. The first peak (m/z 893) produced fragmentation ions at m/z 731 that indicated the loss of a glucose, and m/z 449 corresponding to the loss of a sophorose (324) and *p*-hydroxybenzoic acid (120), and was assigned as cyanidin-3-p-hydroxybenzoyl sophoroside-5-glucoside. The second peak (m/z 949) produced fragmentation ions at m/z 787 (loss of glucose), m/z 449 corresponding to the loss of sophorose (324) and ferulic acid (176) and was therefore assigned as cyanidin 3-(6 –feruloyl-sophoroside)-5-glucoside.

3.2.2. Peonidin derivatives

Peonidin derivatives were identified based in the presence of their characteristic aglycon fragment at m/z 301. Among them, the first molecular ion with m/z 787 was identified as peonidin-3-sophoroside-5-glucoside according to the fragmentation pattern of m/z 625 and 463 that corresponded to the loss of a glucose and sophorose, respectively. Peonidine derivatives acylated with *p*-hydroxy-benzoic acid and caffeic acid were also identified in the present study. In this sense, peonidin-3-*p*-hydroxybenzoylsophoroside-5-*O*-glucoside was identified based upon a molecular ion at m/z 907 and fragmentation ions at m/z 745 that indicated the loss of glucose, and m/z 463 corresponding to the elimination of sophorose and *p*-hydroxybenzoic acid. Another peonidin acylated with caffeic acid and with a molecular ion of m/z 949 was identified as peonidin-3-caffeoyl-sophoroside-5-glucoside based on the fragmentation ions at m/z 787 (loss of a glucose), and m/z 463 (loss of sophorose and caffeic acid). Finally, a diacetylated peonidin derivative with molecular ion m/z 1069 and with a fragmentation pattern of m/z 907 (loss of glucose) and m/z 463 (loss of sophorose and *p*-hydroxybenzoic acid and caffeic acid) was assigned as peonidin 3-6 caffeoyl-6-*p*-hydroxybenzoylsophoroside-5-glucoside (see figure 2B).

3.2.3. Pelargonidin derivatives

Pelargonidin derivatives have also been reported in few varieties of purple sweet potato (Truong et al., 2010; Lee et al. 2013; Xu et al. 2015). In the present study two peaks corresponding to pelargonidin derivatives were identified based in the presence of their characteristic aglycon fragment of m/z 271. A peak with a molecular ion m/z 757 and producing m/z 595 and 433 during MSMS fragmentation (indicative of the loss of a glucose and sophorose, respectively) was identified as pelargonidin 3-sophoroside-5-glucoside. Another peak at m/z 877 and MSMS fragments at m/z 715 and 433 (loss of glucose and sophorose, respectively) was tentatively assigned to pelargonidin 3-p-hydroxybenzoylsophoroside-5-glucoside (see figure 2C). To our knowledge, this is the first study reporting this pelargonidin derivative in *Ipomoea batatas*.

3.2.4. Effect of spray drying on anthocyanins

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Through several years, an important number of purple sweet potato varieties with different anthocyanin content (ranging from 0.3 to 15 mg/g of DM) and profiles were bred and grown for their potential health benefits (Kim et al. 2012; Xu et al. 2015).Our results showed that the content of anthocyanin of purple sweet potato from Piura was within this range but present a relatively low content (1.8 mg/g) compared with other varieties. Peonidin, cyanidin and pelargonidin derivatives, that constituted 45, 34 and 21 % of total anthocyanins respectively, were the predominant anthocyanins present in *Ipomoea batata*s roots which agrees with that previously reported in different varieties (Truong et al. 2010; Lee, Park, Choi & Jung, 2013). Most of them (92,8%) were mono or di acylated (88 and 4,8%, respectively), being 3-*p*-hydroxybenzoylsophoroside the most abundant acylated compound.

Anthocyanins are unstable and easily degraded under variations in temperature and pH conditions, the presence of light and oxygen, among other factors (Hayashi et al. 1996). In the present study, spray drying process greatly reduced (by 92.7%) the content of total anthocyanins. As was indicated for the cinnamoylquinic acids, the high temperatures employed during the spray drying process might explain the low retention of the different anthocyanins identified in the present study. Thermal degradation of *Ipomoea batatas* anthocyanins (10-80 % reduction) under different cooking techniques have been previously reported (Kim et al., 2012; Xu et al. 2015).

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Regarding the spray drying process, in a previous study (Peng et al. 2013), where the retention of total anthocyanins of a sweet potato puree was compared before and after being spray dried, a degradation rate ranging from 15 to 50% was reported. The higher degradation of anthocyanins observed in our study might be explained by the additional losses produced during the pureeing of roots before being spray drying. In this sense, a loss of 30% anthocyanins was reported after 15 minutes to air exposure of cubes roots of purple sweet potato due to the action of polyphenol peroxidase (de Aguiar Cipriano et al. 2015). These authors also reported a higher retention of anthocyanins by the inactivation of this enzyme with a pre-treatment consisting in a combination of heat (less 90ºC) and some inhibiting agents, which was not employed in the present study. Furthermore, the addition of water might also have increased the anthocyanins losses, as was observed in Ipomoea roots when moist heat cooking (steaming, boiling) and dry heat cooking techniques were compared (Kim et al. 2012).

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The impact of spray drying process on the different individual anthocyanins present in *Ipomoea batatas* was not been previously studied. In the present study, the losses seemed to be less pronounced

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for peonidin (89.5% reduction) than for cyanidin and pelargonidin derivatives (98 and 97.8% reduction, respectively). Conversely, some authors (Xu et al. 2015) reported a higher thermal stability of cyanidin than peonidin derivatives, whereas others (Kim et al. 2012) reported a similar response of these anthocyanins when sweet potato was cooked under different techniques. Other factors such as the degree of acylation and the nature of the acyl substituent might also affect the stability of anthocyanins in *Ipomoea batatas* . It is well known that acylation with various phenolic acids make anthocyanins more stable and resistant to heat and light (Hayashi et al. 1996). In this sense, the effect of spray drying on anthocyanins varied with the acylation degree, being those mono acylated less affected (86% reduction) than the ones di and non acylated (98.3 and 98.2 % reduction, respectively). A similar response was also observed during cooking by Xu et al. (2015). These authors also reported a higher thermal stability of anthocyanins acylated with *p*-hydroxybenzoic acid, followed by ferulic acid and caffeic acid acylation. Similarly, in the present study peonidin-3-*p*-hydroxybenzoylsophoroside-5-glucoside seemed to be the most stable anthocyanin.

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3.3. Flavonols

The characterization of flavonol mono and di *O*- glycosides was performed in the positive mode based on the presence of their aglycon quercetin (m/z 303) and kaempferol (287) derived from the losses of glucose (162) and sophorose (324) units during the MSMS fragmentation. The use of standards along with the information of MS fragmentation reported in the literature (Qui et al. 2017), were used to identify the different flavonol structures. Additional information of MSMS fragmentation data acquired in negative mode and the relative presence and intensity of the aglycone fragment was also used to discriminate among 3-*O* and 7-*O* glycosilation position (Qin, et al. 2017). Three peaks releasing MS fragment at m/z 287, were identified as kaempferol derivatives. Two of them presented a similar molecular ion at m/z 611 and were differentiated according to their fragment pattern and elution order. The first one releasing MS fragment ion at m/z 449 and 287, and corresponding to the consecutive loss of two glucose units (162) at different position, was tentatively identified as kaempferol-3,7-di-*O*-glucoside. The second one releasing a unique fragment ion at m/z 287 and compatible to the loss of a sophorose unit (324) was tentatively assigned to kaempferol-3-*O*-sophoroside.

The last peak with a molecular ion at m/z 449 and MS fragment at m/z 287 was identified as kaempferol 3-*O*-glucoside. On the other hand, another two peaks (m/z 627 and 465) releasing a unique MS fragment at m/z 303 were assigned as quercetin derivatives. These peaks were also identified based in their pattern of fragmentation indicating the loss of a sophorose or glucose units as quercetin-3-*O*-sophoroside and quercetin 3-*O*-glucoside respectively.

The presence of quercetin and kaempferol glycosides in leaves and roots of *Ipomoea batatas* has been reported in limited number of studies (Luo et al., 2013; Wang et al. 2018). Among them, Luo et al. 2013 also identified a quercetin dihexose in the leaves of *Ipomoea batatas*. Our results showed that roots also contained a considerable amount of kaempferol-3-*O*-glucoside. As for the other groups of polyphenols, spray drying process reduced greatly the content of flavonols (by 95%).

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3.4. Free aromatic amino acids

Chromatographic conditions used in this study allowed the identification of some free amino acids (AAs) containing aromatic groups such as tryptophan and phenylalanine. The presence of these essential AAs was confirmed and quantified with commercial standards and is reported in table 4.

These AAs, precursor of many constituents of plants including phenolic compounds, are also precursor of neurotransmitter in animals, and in the recent years, a number of important findings related with their involvement on neuropsychiatric conditions have been reported (Strasser, Sperner-Unterwger, Fuchs, & Gostner, 2016). Tryptophan, precursor of serotonine, is one of the most scarce amino acid, and its content in foods is often claimed by exert a positive impact on cognitive and mood. The presence of these free AAs was previously identified in sweet potato roots with distinctive flesh colours (Qiu, Reynolds, Johanningsmeier & Truong, 2020). In the present study spray drying

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process increased the content of phenylalanine. This might indicate a protein hydrolysis with thermal processing, as protein presents a lower thermal stability (below 100ºC) than free AAs, where degradation occurs at temperature above 150º (Rodante & Marrosu, 1990).

3.4. Conclusions

Purple sweet potato root (*Ipomoea batatas L. Lam*) is a good source of anthocyanins and CiQAs derivatives. These compounds are normally separated and identified using different chromatographic conditions and acquisition modes, and consequently only few studies report both phenolic groups simultaneously. In the present study we report that purple root of *Ipomoea batatas* from Peru commonly so-called "Camote morado de Piura" is a source of anthocyanins and caffeoylquinic acids but also contain relevant amounts of feruloylquinic acid derivatives and flavonols. A total of nine anthocyanins including peonidin, cyanidin and pelargonidin glycosylated with sophorose and/or glucose and acylated with caffeic, ferulic and especially with p-hydroxybenzoic acid, were identified in this variety. Twenty nine CiQA, including eight mono-CiQA, fifteen di-CiQA, and five tri-CiQA, were identified on the base of their MS fragmentation profile. Besides the presence of mono and di caffeoyl quinic acids in sweet potato has been widely described, an important number of new CiQAs containing feruloyl moieties in their structures are reported here, to the best of our knowledge, for the first time in *Ipomoea batatas* . Among them, the presence of 3 isomers of FQAs, and 7 isomers of caffeoyl-*O*-feruloylquinic acid which were rarely documented in Ipomoea, accounted for a relevant amount of the total cinnamoylquinic acid content.

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Additionally, the presence of 3 isomers of diFQAs and 5 tri acyl quinic presenting feruloyl moieties identified in this variety were not previously reported in Ipomoea. Finally, purple sweet potato roots also contained flavonols such as kaempferol and quercetin glycosylated with sophorose and/or glucose.

The present study demonstrates that spray drying process of Ipomoea roots caused severe losses (accounting for around 90%) in all the different phenolic groups, including cinnamoylquinic acid, anthocyanin and flavonols. This negative effect is likely to result from a combination of the oxidation produced during the pre-process and the degradation produced by the high temperatures used during the spray-drying process. Among CiQAs, mono CiQAs and in particular those presenting feruloyl moieties were the less affected compounds. Regarding anthocyanins, the negative effect of spray drying seems to be affected by the degree of acylation and the nature of the acyl substituent, being those mono acylated and containing p-hydroxybenoic acid the less affected compounds. Finally, future studies are required to optimize the conditions employed before, and during the spray drying process in order to better preserve the different bioactive substances present in *Ipomoea batatas* roots.

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Fig 1. Extracted ion chromatogram (EIC) of Cinnamoylquinic acid (CiQAs): (A) mono-CiQAs acids; (B) di-CiQAs, (C) tri-CiQAs. 3CQA, 3 caffeoylquinic acid; 5CQA, 5 caffeoylquinic acid; 4CQA, 4 caffeoylquinic acid; 3FQA, 3 feruloylquinic acid; 4 feruloylquinic acid; 5FQA, 5 feruloylquinic acid; 3,4dCQA, 3,4-*O*-dicaffeoylquinic acid; 3,5dCQA, 3,5-*O*-dicaffeoylquinic acid; 4,5dCQA, 4,5-*O*-

Con formato: Sin Resaltar

dicaffeoylquinic acid; 3F4CQA, 3-*O*-feruloyl-4-*O*-caffeoylquinic acid; 3C4FQA, 3-*O*-caffeoyl-4-*O*-feruloylquinic acid; 4F5CQA, 4-*O*-feruloyl-5-*O*-caffeoylquinic acid; 3F5CQA, 3-*O*-feruloyl-5-*O*-caffeoylquinic acid; 3C5FQA, 3-*O*-caffeoyl-5-*O*-feruloylquinic acid; 4C5FQA, 4-*O*-caffeoyl-5-*O*-feruloylquinic acid; 4F5CQA, 4-*O*-feruloyl-5-*O*-caffeoylquinic acid; 3,4dFQA, 3,4-*O*-diferuloylquinic acid; 3,5dFQ, 3,5-*O*-diferuloylquinic acid; 4,5dFQA, 4,5-*O*-diferuloylquinic acid; dCFQA, dicaffeoyl-*O*-feruloylquinic acid ; 3C45dFQA, 3-*O*-caffeoyl-4,5-*O*-diferuloylquinic acid; 34dF5CQA, 3,4-*O*-diferuloyl-5-*O*-caffeoylquinic acid; dCFQA, dicaffeoyl-*O*-feruloylquinic acid

Fig 2. MSMS fragmentation spectrum of anthocyanins. (A) cyanidin-3-*O*-sophoroside-5-*O*-glucoside; (B) peonidin-3-*O*-6-caffeoyl-6-p-hidroxybenzoylsophoroside-5-*O*-glucoside, and (C) pelargonidin-3-p-hidroxybenzoylsophoroside-5-*O*-glucoside. Fragments were indicated as follows: (M, mass; .glu, loss of glucose; -soph, loss of sophorose; -ph, loss of p-hydroxybenzoic acid; -ca, loss of caffeic acid