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GRAPHICAL ABSTRACT

A comparative study on the phenolic bioaccessibility, antioxidant and inhibitory effects on carbohydrate-digesting enzymes of maca and mashua powders

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ABSTRACT

The polyphenolic content of dehydrated maca (MA) and mashua (M) powders, promising functional ingredients, and their antioxidant activity and inhibition of carbohydrate digestion was evaluated following *in vitro* gastrointestinal digestion (GID). Moreover, the impact of steaming on bioaccessibility and bioactivity of the M phenolic fraction was investigated. MA phenolic fraction composed mainly of caffeic-4-*O*-glucosides showed high bioaccessibility (117.8%), antioxidant activity (142.9 μmol Trolox equivalents, TE/g) and weak effectiveness to inhibit carbohydrate digestion after GID. Anthocyanins were the major compounds in the M phenolic fraction with low bioaccessibility (22.4%), although a superior antioxidant activity (446.7 μmol TE/g) and modest inhibitory effects on carbohydrate digestion after GID. Generally, bioaccessibility and bioactivity of M decreased after steaming process. MA and M powders contain appreciable concentrations of phenolics, although M polyphenols were more effective on modulating carbohydrate digestion.

Keywords: bioactivity, bioaccessibility, *in vitro* digestion, mashua, maca, phenolic compounds;

1. Introduction

Hyperglycemia is associated to developing type-2 diabetes mellitus due to increased generation of intracellular reactive oxygen species that subsequently augment the risk of insulin resistance (Yan, 2014). Currently, a beneficial strategy in the prevention of certain metabolic disorders is to slow down dietary carbohydrates digestion and attenuate oxidative stress through dietary compounds (Houghton, Kerimi, Tumova, Boyle, & Williamson, 2018; Putnik, Bezuk, Barba, Lorenzo, Polunic, & Bursac, 2020). The beneficial effects of dietary polyphenols in connection with glycaemic control have become an area of interest. Several human studies have demonstrated that phenolic compounds have positive implications improving overall glucose homeostasis and postprandial glycaemic control (Costa et al., 2017). Inhibition of salivary and pancreatic α -amylases and intestinal brush border glucosidases have been identified among the mechanisms of action explaining the protective effects of polyphenols against hyperglycaemia (Costa et al., 2017).

Maca (*Lepidium meyenii* Walp.) and mashua (*Trapaecolum tuberosum* Ruiz & Pavón) are tuber crops belonging to *Brassicaceae* and *Tropaeolaceae* families, respectively. These tubers, native from the Andean region, have been used for centuries in traditional medicine (Campos, Chirinos, Gálvez Ranilla, & Pedreschi, 2018). In the last years, maca and mashua have been considered as valuable sources for functional food development due to their excellent nutritional and nutraceutical properties (Apaza-Ticona, Tena-Pérez, & Bermejo-Benito, 2020). Both Andean tubers are rich in starch, fiber, proteins, vitamins and minerals, and contain significant amounts of phytochemicals (Campos, Chirinos, Gálvez-Ranilla, & Pedreschi, 2018; Wang & Zhu, 2019). Phenolic compounds are among the bioactive constituents of maca and mashua (Wang & Zhu, 2019). The main phenolic compounds present in maca are flavonolignans, phenolic acids and flavanols (Bai, He, Roller, Lai, Bai, & Pan, 2015; Yabar, Chirinos, & Campos, 2019). Mashua tuber

is characterized by the presence of free and conjugated hydroxybenzoic and hydroxycinnamic acids, monomeric and oligomeric flavan-3-ols, flavonols and anthocyanins (Chirinos, Campos, Costa, Arbizu, Pedreschi, & Larondelle, 2008).

Maca and mashua tubers are found in market as food supplements commercialized as dried powders (Apaza-Ticona et al., 2020). However, little is known regarding the ability of maca and mashua in the form of dehydrated powders to deliver phenolics in the gut able to exert antioxidant effects and inhibit dietary carbohydrate digestion. Given the great differences in the phenolic profile of both tubers it is interesting to study the bioaccessibility of the specific phenolic classes occurring in both tubers and relate these changes to their potential efficacy to control glycemic response. Hence, preliminary *in-vitro* studies are needed to determine the bioaccessibility and bioactivity of maca and mashua polyphenols. Processing and bioaccessibility (proportion of the ingested compounds that are released from the food matrix along the digestive system) represent the major factors affecting the phenolic composition and bioactivity of foods in the gastrointestinal tract (Carvalho-Tavares et al., 2019). Therefore, the objective of our study was to characterize the nutritional and phenolic composition from dehydrated powders from black genotypes of maca and mashua tubers (Figure 1) and study its bioaccessibility, antioxidant activity and inhibition of carbohydrates digestion following *in vitro* gastrointestinal digestion (GID). To increase the storage stability and bioaccessibility of phenolics in dehydrated foods, different thermal pre-treatments have been explored prior dehydration process, although effects were highly variable depending on type and extent of processing conditions (Yu et al., 2019). The influence of applying steam prior dehydration process on the bioaccessibility and bioactivity of phenolic compounds of mashua was also assayed.

2. Materials and Methods

2.1. Plant material

Gelatinized black maca powder (MA) and black mashua tubers were purchased from Amazon Andes (Lima Perú) and Peruvian Organic Foods (Junín Perú), respectively. Three commercial batches of maca powder and mashua tubers were used in this study. Mashua tubers were washed with tap water and freeze-dried for 5 days at -80 °C and -0.001 mBa (Labconco, Madrid, Spain). Moreover, mashua tubers were steamed in triplicate over boiling water for 30 min using a Thermomixer (Vorwerk, Germany) cooking robot before freeze-drying in the conditions mentioned above. Dehydrated mashua (M) and steamed mashua (SM) tubers were milled using an Ika grinding system (Staufen, Germany) and the resulting powder was passed through a 0.3 mm sieve. Powders were packed using vacuum-sealed plastic bags and stored at -20 °C.

2.2. In vitro gastrointestinal digestion

MA, M and SM were digested as previously reported (Minekus et al., 2014). Briefly, samples (3g) were digested in simulated salivary fluid (pH 7.0) containing 75 U/mL human salivary α -amylase type IX-A (EC 3.2.1.1, Sigma-Aldrich, Madrid, Spain) for 2 min at 37 °C. Subsequently, gastric fluid (pH 3.0) containing 2000 U/mL pepsin (EC 3.4.23.1, Sigma-Aldrich) was added and incubated at 37 °C in an orbital shaker at 150 rpm for 120 min. Afterwards, simulated intestinal fluid containing 100 U trypsin activity/mL pancreatin (Sigma-Aldrich) and bile extract (10 mM in the final mixture) is added and incubated at 37 °C in an orbital shaker at 150 rpm for 120 min. Digestions were performed in duplicate. Sample aliquots were withdrawn at the starting point (negative control) and after gastric (120 min) and intestinal digestion (240 min) and enzymatic activity was immediately inactivated using 5 mmol/L Pefabloc (Sigma-Aldrich). All samples were freeze-dried and stored at -20 °C until analysis.

2.3. Extraction of phenolic compounds

Phenolics extraction was performed using 0.5 g of powdered samples and 10 mL of 80% acidified methanol containing 0.1% hydrochloric acid at room temperature for 10 min under magnetic stirring. Sample suspensions were centrifuged at 10,000 x *g* and 5 °C for 10 min. Combined supernatants were evaporated to dryness at 40 °C in a Buchi R-215 rotatory evaporator (Flawil, Switzerland). Dry extract was dissolved in 1 mL of 80% methanol and filtered through 0.22 µm membrane (Agilent, Santa Clara, CA, USA) for HPLC analysis.

2.4. HPLC-ESI-UV-MS/MS analysis of phenolic compounds

Analyses were carried out as described previous by García-Villalva, Espín and Tomás-Barberán (2016). An Agilent HPLC 1100 system with a diode array detector (DAD) coupled with a Bruker Daltonics ion trap mass spectrometer (MS) detector equipped with electrospray ionization source acting in negative mode was used. Samples were injected (10 µL) into a reverse phase C18 Kinetex-F5 column (5 µm, 250 × 4.6 mm; Phenomenex, Macclesfield, UK) and compounds elution was carried out at room temperature, flow rate of 0.8 mL/min using as mobile phases 1% formic acid (solvent A) and acetonitrile (solvent B) following a gradient profile of 0–5 min, 3% B; 5–15 min, 9% B; 15–45 min, 16% B; 45–47 min, 50% B; 47–52 min, 90% B; 52–57 min, 3% B and then return to initial conditions. The detection wavelengths in DAD of flavonoids, phenolic acids and anthocyanins were 280 nm, 320 nm and 520 nm, respectively. MS parameters were nebulizing gas (nitrogen) pressure of 65 psi, flow rate of 11 L/min at 350 °C. The capillary voltage was 3.5 kV. Mass scan and daughter spectra were recorded in the range of *m/z* 100-1200. Collision-induced fragmentation was carried out in the ion trap with helium and a collision energy set at 50%. Retention time, molecular mass and fragmentation pattern were used to identify phenolic compounds. Quantification was made by external calibration curves in the concentration range between 5 and 500 µg/mL of standard compounds: caffeic acid, delphinidin-

3-*O*-rutinoside chloride, (+)-catechin, (-)-gallic acid, rutin, myricetin-3-*O*-glucoside, procyanidin dimer B2, kaempferol-3-*O*-rutinoside, luteolin-7-*O*-glucoside (Extrasynthese, Lyon, France). Analysis were performed in duplicate and results were reported as mg/g of soluble fraction in dry weight basis (dw).

The bioaccessibility of phenolic compounds refers to the amount of phenolic compounds released from the food matrix after GID that could become available for absorption into the systemic circulation. Bioaccessibility was calculated as shown in *Equation 1*:

$$\text{Bioaccessibility (\%)} = (A / B) \times 100 \quad (\text{Equation 1})$$

where A is the total phenolic content (mg/g dw) in samples after GID and B is the total phenolic content (mg/g dw) in samples before GID.

2.5. Determination of oxygen radical absorbance capacity

Oxygen radical absorbance capacity (ORAC) was determined by fluorescence as previously reported (Torino et al., 2013). ORAC values were obtained by external calibration using Trolox as standard and a linear concentration range (0-160 μ M). Analysis were performed in duplicate and data were reported as μ mol of Trolox Equivalents (TE)/g of sample on dw basis.

2.6. Determination of α -amylase inhibitory activity

The α -amylase inhibition assay was carried out as reported previously (Vilcacundo, Martínez-Villaluenga, & Hernández-Ledesma, 2017). Percent inhibition was calculated as shown in *Equation 2*:

$$\text{Inhibitory activity (\%)} = [(Abs_{\text{sample}} - Abs_{\text{control}}) / Abs_{\text{control}}] * 100 \quad (\text{Equation 2})$$

Concentration of samples that inhibit 50% of original enzyme activity (IC_{50} value) was calculated from non-linear regression sigmoidal dose-response curves using GraphPad Prism 6.00 (San Diego, CA, USA).

2.7. Determination of α -glucosidase inhibitory activity

Sucrase and maltase inhibitory activities were assessed by measuring the inhibition of glucose released from the hydrolysis of sucrose and maltose, respectively. Assays were carried out according to Vilcacundo et al. (2017). Percent inhibition was calculated as shown in Equation 3:

$$\text{Inhibitory activity (\%)} = \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}})}{\text{Abs}_{\text{control}}} * 100 \quad (\text{Equation 3})$$

Concentration of samples that inhibit 50% of original enzyme activity (IC_{50} value) was calculated from non-linear regression sigmoidal dose-response curves using GraphPad Prism 6.00 (CA, USA).

2.8. Statistical analysis

Results were expressed as the mean of three replicates $\pm$ standard deviation. Statistical analysis were carried out using Statgraphics Plus software, version 5.1 (Statistical Graphics Corporation, Rockville, MD, USA). The significance of differences between groups of treatments was analysed by one-way analysis of variance (ANOVA) and post hoc Duncan's multiple range test ($p \leq 0.05$).

3. Results and Discussion

3.1. Phenolic profiles of maca and mashua powders

Chromatographic and mass spectral data is shown in Supplementary Tables S1 and S2. Important differences in the content and major groups of phenolic compounds in MA (Table 1)

and M (Table 2) were observed. Total individual phenolic content (TIPC) was defined as the sum of all phenolics quantified by chromatography, expressed in mg/g dw. TIPC in MA was 2.7 mg/g dw (Table 1), with caffeic acid glucosides being the predominant phenols (71.3% of TIPC), followed by much lower amounts of luteolin-7-*O*-6"-malonylglucoside (14.3% of TIPC), kaempferol rutinoside (7% of TIPC) and catechin derivative (3.7% of TIPC). A different phenolic profile was recently reported by Yabar et al. (2019) who observed flavan-3-ol derivatives as the most abundant polyphenols in dried MA roots at harvest (84.2% of TIPC) followed by hydroxybenzoic acid derivatives (15.7% of TIPC). These differences in the phenolic profile of dried MA could be related to extrinsic factors during postharvest processing (Yabar et al., 2019).

Regarding M, a higher TIPC (16.4 mg/g powder) compared to MA was observed ($p < 0.05$). Anthocyanins were the major phenolic compounds, namely non-acylated and acylated 3,5-diglucoside derivatives of delphinidin (21% and 55.1% of TIPC, respectively), followed by galocatechin derivatives and aglycone (13.7% and 6.8% of TIPC). Caffeic acid glucosides (3.6%), rutin (0.7%), myricetin-3-glucoside (0.7%) and a procyanidin dimer (0.7%) were detected as minor phenolic compounds. To the best of our knowledge, this is the first study showing the anthocyanin profile of black M. The non-anthocyanin phenolic profile of the present study showed some similarities to that reported by Chirinos et al. (2008) for purple M genotypes. In this latter study, phenolic profile was composed mainly by derivatives of *p*-hydroxybenzoic, galocatechin, cinnamic acid and epigallocatechin followed by minor amounts of procyanidin B2, myricetin derivative, rutin, epicatechin derivative, *p*-coumaric and *o*-coumaric derivatives.

3.2. Effect of steaming on the phenolic profile of mashua powder

The analysis of individual phenolic compounds showed different responses to the steaming process of M. After thermal treatment, there was a significant reduction of the TIPC in M (55%).

Likewise, Yu et al. (2019) observed a loss of 54% of TIPC in mulberry (*Morus alba* L.) leaves after steaming. In the present study, the highest losses were observed for non-acylated and acylated 3,5-diglucoside anthocyanins (64 and 73%, respectively) with a consequent increase in gallocatechin derivatives and aglycone by 30.6% and 16%, respectively. Similar to our results, Su et al. (2019) observed that thermal treatments at 121 °C of lychee juice promote the generation of (-) gallocatechin. The physicochemical mechanism behind this effect is linked to thermal-induced hydrolysis of unstable compounds such as procyanidin polymers, leading to the release of dimers and monomers (Alongi, Verardo, Gorassini, & Anese, 2018). Our results are also consistent with the literature showing that thermal processing reduced the levels of anthocyanins in foods due to thermal degradation and oxidation pathways (Patras, Brunton, O'Donnell, & Tiwari, 2010). Carvalho-Tavares et al. (2019) observed a significant reduction of total anthocyanins in juice by application of saturated steam (2.5 h) to BRS Violeta grapes.

3.3. Bioaccessibility of phenolic compounds in maca and mashua powders

Evaluation of bioaccessibility of phenolic compounds is the first step estimating the amount that might be available for absorption in the gastrointestinal tract. TIPC in MA increased after GD (39.4%) and GID (17.8%), respectively, with respect to non-digested MA (Table 1). This fact could indicate that the *in vitro* digestion releases phenolic compounds from the food matrix as consequence of enzymatic activity as well as acid conditions in GD that might enhance breakage of covalent or non-covalent bounds between polyphenols and polysaccharides, proteins, etc. Similar effects of GID on phenolics bioaccessibility have been recently reported for purple sweet potato (Meng, Tan, & Feng, 2019).

The bioaccessible phenolic fraction of MA in the stomach was composed of caffeic acid glucosides as the most abundant compounds followed by luteolin malonyl glucoside, kaempferol

rutinoside and catechin derivative (Table 1). A significant decrease in the bioaccessibility of luteolin malonyl glucoside, keampferol rutinoside and catechin derivative was observed after GID with respect to GD ($p<0.05$). These results indicate that intestinal conditions (alkaline pH, pancreatin and bile salts) are negatively affecting the stability of some phenolic compounds as previously reported (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010).

On the contrary, GID decreased the concentration of the main phenolic compounds present in M, reaching at the end of GID the lowest concentration ($p<0.05$, Table 2). Non-acylated and acylated delphinidin derivatives showed the lowest bioaccessibility after GD (46.5 and 28.4%, respectively) and GID (1.4 and 0.4%, respectively). The stronger hydrophobic interactions of acylated anthocyanins with biopolymers (Deineka, Makarevich, & Deineka, 2018) could provide an explanation for their lower bioaccessibility compared to non-acylated counterparts. Our results demonstrated a high variability on the stability of different compounds to gastrointestinal conditions in agreement with other studies (Sadeghi-Ekbatan et al., 2016). Low bioaccessibility of anthocyanins observed after GID could be explained by their chemical instability of flavylum cation structure at pH values over 4.5, leading to the chalcone form at slightly alkaline conditions (pH 7.5) (Nielsen, Haren, Magnussen, Dragsted, & Rasmussen, 2003). Similarly, other authors have reported a low bioaccessibility of anthocyanins after GID of foods (Correa-Betanzo, Allen-Vercoe, McDonald, Schroeter, Corredig, & Paliyath, 2014; Ryu & Koh, 2018). Correa-Betanzo et al. (2014) observed nearly 10–15% reduction of the original levels of blueberry anthocyanins after overall digestion process.

Bioaccessibility values of gallocatechin derivatives and aglycone, rutin, myricetin-3-rutinoside and procyanidin dimer were high under gastric conditions ranging from 86.9% to 116.8% (Table 3). However, after intestinal digestion polyphenols were significantly reduced in M, with gallocatechin derivatives (33.9%) and procyanidin dimer (57.3%) being the less

bioaccessible. Decreased concentration of flavonoids during digestion process could be due to the interaction with other food constituents (polysaccharides, protein and amino acids) and instability under the alkaline intestinal conditions that might induce oxidation, polymerization and other chemical transformations (Lucas-Gonzalez, Navarro-Coves, Pérez-Álvarez, Fernández-López, Muñoz, & Viuda-Martos, 2016). Similar to MA, caffeic acid glucosides increased in the overall digestion process with respect to their initial concentration in non-digested M.

Regarding bioaccessibility of phenolic compounds in SM, 50% of non-acylated and acylated anthocyanins were bioaccessible in the gastric phase, values slightly higher than those reported for M (Table 2, $p<0.05$). On the other hand, bioaccessibility values for the sum of galocatechin derivatives and aglycone, and procyanidin dimer decreased significantly in the stomach with respect to M ($p<0.05$). No statistical differences were observed for bioaccessibility of the rest of polyphenols in SM compared to M (Table 2). In the intestinal phase, bioaccessibility of SM phenolic fraction (15.8%) was reduced with respect to GD ($p<0.05$) and it was significantly lower than the value observed in M. In the intestine, bioaccessible fraction of SM was uniquely composed by galocatechin derivatives and caffeic acid glucosides while anthocyanins, galocatechin, myricetin-3-rutinoside and procyanidin dimer were not detected in this step of digestion.

The above results confirmed a negative influence of steaming on the bioaccessibility of M polyphenols. This trend is consistent with previous reports showing that steaming reduced bioaccessibility of 3-caffeoylquinic acid, rutin, isoquercitrin, quercetin-malonyl-glucoside and astragalin in mulberry leaves (Yu et al., 2019). The lower bioaccessibility for total phenolics observed after thermal processing could be due to the combination of several factors including interaction with other food matrix components, changes in solubility, thermal and oxidative degradation (Lucas-González et al., 2018). While present results appear to demonstrate the

negative impact of steaming on phenolic bioaccessibility of M, several limitations of the current study do suggest that some caution should be applied in the interpretation. The thermal treatment duration of this study is critical to consider. Recent results reported by Li, Hao & Zhu (2018) have demonstrated that short time steaming (1 min) increased total bioaccessible phenolic compounds of radish sprouts. This suggest a shorter steaming time would have been required to improve the recovery of phenolics in M and make them more available to impact potential consumer health outcomes. Several points need to be considered in future studies. Given that differential effects of steaming in phenolic bioaccessibility seems to depend on processing conditions (pressure, temperature and time), a wise approach would be based on mathematical modelling and multiple response optimization to identify the operational conditions that maximize the phenolic bioaccessibility and functional properties of M powder. The application of non-thermal treatments such as ultrasounds would be a possible useful tool in enhancing the bioaccessibility of M phenolic compounds.

3.4. Antioxidant capacity of maca and mashua powders: Effect of steaming and GID

In consistency with TPC, higher ORAC values were observed for M samples (158 $\mu\text{mol TE/g dw}$) compared to MA (73.8 $\mu\text{mol TE/g dw}$) (Table 3 and 4, respectively). Our results are complementary to those reported for MA methanolic extracts using complementary antioxidant assays such as ferric reducing power and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (Lee & Chang, 2019). Regarding M, Chirinos et al. (2008) showed a high genetic variability for ORAC values of different genotypes of M that ranged from 7.9 to 60.5 $\mu\text{moles TE/g fresh weight}$. Considering that moisture content in fresh mashua tubers was 90% (data not shown), ORAC value reported in the present study for M was within the range reported by Chirinos et al. (2008). Steaming caused a significant increase in the antioxidant capacity of M

($p < 0.05$), likely because thermal-induced formation of Maillard reaction products with antioxidant properties (Carvalho-Tavares et al., 2019).

In this study, the antioxidant capacity of MA and M polyphenols during the *in vitro* digestion process was also evaluated. Gastric and intestinal phases have a profound effect on antioxidant activity of MA and M. Compared to the initial antioxidant activity, ORAC values for MA and M increased significantly during GID (Table 3 and 4, respectively). A similar trend was found in earlier studies for *in vitro* digested pomegranate peel where an increase in the antioxidant capacity was reported throughout the digestion steps compared to non-digested sample (Gullon, Pintado, Barber, Fernández-López, Pérez-Álvarez, & Viuda-Martos, 2015). Similarly, Sadeghi-Ekbatan et al. (2016) observed a higher antioxidant capacity (measured by the 2,2'-azino-bis[3-ethylbenzothiazoline-6-sulphonic] acid [ABTS] assay) of a mixture of pure polyphenols in all gastrointestinal compartments of a dynamic multistage gastrointestinal model (Sadeghi-Ekbatan, et al., 2016). In addition to phenolics contribution to antioxidant activity during GID, the liberation of other antioxidants (ascorbates, carotenoids, tocopherols, etc) from food matrices have been proposed as explanation for the increased antioxidant activity of different plant food products (Gullon et al., 2015).

The transition of gastric to intestinal conditions reduced significantly ORAC values for MA (17%), M (26%) and SM (43%) ($p < 0.05$). Similarly, Lucas-Gonzalez et al. (2016) observed 12.5% decrease in the antioxidant capacity (determined by DPPH assay) of maqui berry after GD and 75.4% reduction after GID with respect to non-digested sample. Antioxidant activity of gastric and intestinal digests of SM was far below that found in M digests, although ORAC values remained higher compared to non-digested sample. The antioxidant activity of sample extracts depend on the composition and the concentration of its antioxidants, mainly phenolic

compounds, therefore, the lower antioxidant potential of SM during the GID might be related to the lower amount of bioaccessible phenolic compounds.

3.5. The α -amylase and α -glucosidase inhibitory activities: Effect of steaming and GID

MA was able to inhibit pancreatic α -amylase as well as sucrase and maltase activities of intestinal α -glucosidases (IC_{50} = 3.3, 2.8 and 3.6 mg/mL, respectively). Similarly, M inhibited the three enzymatic activities (α -amylase, maltase and sucrase) although with a higher potency compared to MA (IC_{50} = 2.1, 0.8 and 1.2 mg/mL, respectively). The differences between the effectiveness of MA and M on inhibition of digestive carbohydrases could be related not only to phenolic concentration but also to phenolic profile. Previous studies have shown that phenolic acids are only very weak inhibitors of carbohydrate-digesting enzymes compared to anthocyanins and other flavonoids (Nyambe-Silavwe & Williamson, 2018). In addition, potatoes rich in anthocyanins, tended to have reduced glucose responses and glycaemic index values compared to yellow and white varieties, effect that was partially attributed to inhibition of intestinal α -glucosidases and glucose release in the gastrointestinal tract (Ramdath, Padhi, Hawke, Sivaramalingam, & Tsao, 2014). Thus, all these findings may explain the higher effectiveness of M with anthocyanins as the dominant compounds of phenolic fraction compared to MA in which phenolic acids were the major compounds.

As compared to acarbose, a drug also used for the pharmacological treatment in the management of type-2 diabetes with IC_{50} values of 0.16, 0.03 and 0.07 mg/mL for α -amylase, sucrase and maltase activities, respectively (González-Montoya, Hernández-Ledesma, Mora-Escobedo, & Martínez-Villaluenga, 2018), MA and M extracts could be considered modest inhibitors of carbohydrate-digesting enzymes.

SM showed higher IC_{50} values for inhibition of carbohydrate-digesting enzymes with respect to M (Table 3), indicating that steaming reduced the effectiveness of M to inhibit *in vitro* carbohydrate digestion. These results were expected since SM had lower amount of accessible phenolics compared to M.

Regarding GD, it was observed that α -amylase ($IC_{50} = 3.8$ mg/mL) and sucrase inhibitory activities of MA remained unchanged in the gastric phase ($IC_{50} = 3.4$ mg/mL), with the exception of maltase inhibitory activity that was not detected (Table 3). After GID, inhibitory potency of MA was significantly reduced ($p < 0.05$), as demonstrated by the higher IC_{50} values with respect to GD. In the case of M, the potential to inhibit carbohydrate-digesting enzymes was significantly reduced throughout the time course of digestion process, with this decrease being significantly higher in the intestine environment with respect to gastric conditions ($p < 0.05$). Similar effect of GID on inhibition of α -amylase and α -glucosidases was observed for SM, although this sample was by far less active than M, inhibiting only intestinal sucrase activity at the end of the overall digestion.

CONCLUSIONS

This work represents a contribution to the analysis of the scarcely studied Andean MA and M tubers and provides additional information concerning the stability of phenolic compounds from these new food sources and their biological activity to thermal treatments and digestion process. Products assayed in this study contained appreciable concentrations of phenolics that include mainly highly bioaccessible phenolic acids in MA and low bioaccessible anthocyanins in M. MA and M phenolics exerted antioxidant and inhibitory effects on starch, maltose and sucrose digestion although more limited at the end of intestinal phase. As compared to MA, M powder exhibited higher antioxidant and inhibitory activity against carbohydrate-digesting enzymes

although steaming reduced the phenolics bioaccessibility and its potential to modulate carbohydrate digestion. While the potential antioxidant activity and inhibition of the α -amylase and α -glucosidases by M phenolics is more promising than that for MA, its *in vivo* impact on glycaemic control requires further evaluation. These results may contribute to promote the use of these tubers for food production and in the last term to conserve biodiversity in food systems.

Acknowledgements

This study was funded by grants AGL2017-83718-R (AEI/FEDER, UE) and AGL2015-66886-R (MINECO, FEDER/UE). We express our gratitude to B. Castro for technical assistance. L. Paucar-Menacho acknowledges to Consejo Nacional de Ciencia y Tecnología (CONCYTEC, Peru) for financial support (Grant number 193-2018-FONDECYT).

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Table 1. Content (mg/g dw) and bioaccessibility (%) of phenolic compounds before and after gastric and gastrointestinal digestion of black maca powder

Compound	Non-digested	GD		GID	
	mg/g	mg/g	% ¹	mg/g	% ¹
Caffeic acid 4- <i>O</i> -glucoside	1.31±0.05 ^b	1.59±0.00 ^a	121.30±11.97 ^A	1.69±0.12 ^a	128.94±9.25 ^A
Caffeic acid 4- <i>O</i> -glucoside	0.73±0.01 ^b	1.00±0.05 ^a	137.36±6.71 ^B	1.15±0.09 ^a	159.25±11.81 ^A
Luteolin-7- <i>O</i> -(6''malonylglucoside)	0.39±0.03 ^b	0.78±0.07 ^a	197.43±13.80 ^A	0.09±0.02 ^c	22.62±5.81 ^B
Kaempferol rutinoside	0.19±0.02 ^a	0.26±0.07 ^a	137.15±28.35 ^A	0.18±0.04 ^a	94.74±23.25 ^A
Catechin derivative	0.10±0.03 ^a	0.16±0.01 ^a	114.29±19.02 ^A	0.09±0.02 ^a	89.16±21.88 ^A
TIPC	2.72±0.14 ^b	3.76±0.35 ^a	139.38±9.65 ^A	3.20±0.29 ^a	117.8±10.85 ^A

* Mean ± standard deviation of three replicates. Different lowercase letters within a row indicate significant differences ($p \leq 0.05$) among phenolic content of non-digested and digested samples. Different uppercase letters within a row indicate significant differences ($p \leq 0.05$) between bioaccessibility values at each digestion step.

GD: gastric digestion; GID: gastrointestinal digestion; nd: not detected; TIPC: Total individual phenolic content

¹Values are percentage with respect to the TIPC recovered after each digestion phase

Table 2. Content (mg/g dw) and bioaccessibility (%) of phenolic compounds before and after gastric and gastrointestinal digestion of mashua and steamed mashua powder*

Compound	M					SM				
	Non-digested (mg/g)	GD		GID		Non-digested (mg/g)	GD		GID	
		mg/g	% ¹	mg/g	% ¹		mg/g	% ¹	mg/g	% ¹
dp-3-sph-5-rhm 1	0.68±0.03 ^a	0.24±0.01 ^b	35.20±1.74	nd	-	0.21±0.00 ^{a*}	0.11±0.01 ^{b*}	53.39±6.58*	nd	-
dp-3-sph-5-rhm 2	0.46±0.02 ^a	0.19±0.01 ^b	40.65±1.31	nd	-	0.20±0.02 ^{a*}	0.12±0.01 ^{b*}	61.10±3.37*	nd	-
dp-3-glc-5-rhm 1	0.76±0.05 ^a	0.43±0.01 ^b	56.34±0.92 ^A	0.02±0.00 ^c	2.22±0.60 ^B	0.41±0.02 ^{b*}	0.20±0.02 ^{c*}	48.66±4.00*	nd	-
dp-3-glc-5-rhm 2	0.68±0.03 ^a	0.26±0.02 ^b	37.87±2.31 ^A	0.02±0.00 ^a	3.57±0.74 ^B	0.43±0.02 ^{b*}	0.26±0.02 ^c	60.30±3.96*	nd	-
dp-3-glc-5-rhm 3	0.48±0.00 ^a	0.31±0.00 ^b	64.75±0.00	nd	-	nd	nd	nd	nd	-
SUM²	3.45±0.09^a	1.42±0.00^b	46.46±0.10	0.04±0.01	1.35±0.31	1.24±0.02^{a*}	0.69±0.01^{b*}	55.80±1.06*	nd	-
dp-3-glc-5-acrhm 1	0.47±0.02 ^a	0.18±0.01 ^b	37.53±2.89	nd	-	0.27±0.01 ^{a*}	0.13±0.01 ^{b*}	49.59±4.89*	nd	-
dp-3-glc-5-acrhm 2	0.82±0.06 ^a	0.31±0.01 ^b	38.42±1.22	nd	-	0.41±0.01 ^{a*}	0.24±0.02 ^{b*}	57.52±3.72*	nd	-
dp-3-glc-5-acrhm 3	0.66±0.00 ^a	0.25±0.01 ^b	38.09±1.12	nd	-	0.21±0.00 ^{a*}	0.11±0.01 ^{b*}	54.88±6.98*	nd	-
dp-3-glc-5-acrhm 4	1.86±0.09 ^a	0.65±0.01 ^b	35.16±2.90 ^A	0.02±0.00 ^c	1.15±0.05 ^B	0.47±0.01 ^{a*}	0.24±0.01 ^{b*}	50.50±2.78*	nd	-
dp-3-glc-5-acrhm 5	4.52±1.98 ^a	0.92±0.09 ^b	20.45±1.95 ^A	0.02±0.00 ^c	0.36±0.03 ^B	0.68±0.01 ^{a*}	0.31±0.00 ^{b*}	45.46±0.00*	nd	-
dp-3-glc-5-acrhm 6	0.72±0.00 ^a	0.25±0.01 ^b	35.06±1.27	nd	-	0.36±0.01 ^{a*}	0.26±0.11 ^b	73.72±30.45*	nd	-
SUM³	9.04±1.84^a	2.57±0.15^b	28.43±1.71^A	0.04±0.00^c	0.41±0.03^B	2.41±0.01^{a*}	1.30±0.08^{b*}	53.99±3.29*	nd	-
glcat d1	0.12±0.01 ^c	0.28±0.01 ^a	144.63±5.96 ^B	0.18±0.01 ^b	233.36±1.38 ^A	0.36±0.01 ^{a*}	0.43±0.10 ^{a*}	120.80±26.70 ^A	0.23±0.02 ^{a*}	64.23±6.43 ^{B*}
glcat d2	0.95±0.07 ^a	0.68±0.09 ^b	71.56±9.45 ^A	0.13±0.01 ^c	14.15±1.48 ^B	1.04±0.00 ^{a*}	0.12±0.00 ^{c*}	11.58±0.12 ^{B*}	0.26±0.01 ^{b*}	25.09±0.66 ^{A*}
glcat d3	0.41±0.01 ^a	nd	nd	0.07±0.00 ^b	18.05±1.10	1.19±0.01 ^{a*}	0.58±0.02 ^a	48.49±2.06 ^A	0.07±0.01 ^b	6.08±1.03 ^{B*}
glcat d4	0.18±0.00 ^b	0.43±0.00 ^a	236.63±0.48 ^A	0.13±0.02 ^b	68.91±13.29 ^B	0.09±0.02 ^{b*}	0.13±0.01 ^{b*}	138.72±11.00 ^{A*}	0.27±0.02 ^{a*}	79.11±8.49 ^B
glcat d5	0.11±0.00 ^a	0.12±0.00 ^a	109.67±3.17 ^A	0.08±0.01 ^a	74.31±13.31 ^B	0.16±0.00 ^{a*}	0.10±0.04 ^a	60.59±25.24 ^{A*}	0.12±0.00 ^{a*}	76.30±2.42 ^A
glcat d6	0.07±0.01 ^a	0.07±0.01 ^a	105.71±19.50 ^A	0.07±0.00 ^a	102.36±6.03 ^A	0.10±0.01 ^{a*}	0.13±0.01 ^{a*}	133.72±11.04 ^{A*}	0.07±0.02 ^b	74.54±21.73 ^A
SUM⁴	2.25±0.06^a	1.96±0.07^b	86.97±3.30^A	0.76±0.03^c	33.88±1.34^B	2.94±0.05^{a*}	1.75±0.29^b	59.55±5.83^{A*}	0.77±0.01^c	26.03±0.31^{B*}
Ca 4-O-glc 1	0.29±0.01 ^b	0.19±0.01 ^c	64.74±2.95 ^B	0.79±0.01 ^a	269.47±4.28 ^A	0.22±0.00 ^{a*}	0.29±0.12 ^{a*}	133.23±52.63 ^{A*}	0.26±0.01 ^{a*}	118.71±2.45 ^{A*}
Ca 4-O-glc 2	0.30±0.00 ^c	0.50±0.01 ^b	168.39±4.24 ^B	0.83±0.06 ^a	276.63±21.02 ^A	0.36±0.01 ^{a*}	0.38±0.01 ^{a*}	107.76±0.00 ^{A*}	0.22±0.03 ^{b*}	63.36±7.18 ^{B*}
SUM⁵	0.59±0.01^c	0.69±0.02^b	116.82±3.60^B	1.62±0.00^a	274.08±12.70^A	0.57±0.00^a	0.67±0.10^a	117.46±17.44^A	0.36±0.14^{b*}	83.82±5.38^{B*}
glcat	1.12±0.06 ^a	1.02±0.14 ^a	90.88±12.11 ^A	0.94±0.11 ^a	83.61±10.22 ^A	1.30±0.01 ^a	0.64±0.03 ^{b*}	49.37±2.10*		
rut	0.11±0.01 ^a	0.12±0.0 ^a	106.60±7.93 ^A	0.08±0.01 ^b	73.21±11.50 ^B	0.15±0.00 ^a	0.14±0.03 ^a	93.68±17.78	nd	-
myr-3-rut	0.12±0.00 ^a	0.14±0.02 ^a	114.85±16.10 ^A	0.13±0.02 ^a	108.31±16.55 ^A	0.11±0.01 ^a	0.13±0.01 ^a	116.05±11.09	nd	-

Pro dimer	0.12±0.00 ^a	0.12±0.00 ^a	97.94±3.19 ^A	0.07±0.00 ^b	57.53±0.82 ^B	0.15±0.01 ^a	0.12±0.00 ^b	81.38±0.00*	nd	-
TIPC	16.41±1.64^a	8.03±0.40^b	48.95±2.41^A	3.68±0.16^c	22.44±1.00^B	8.88±0.07^a	5.46±0.35^{b*}	61.47±3.98^{B*}	1.44±0.06^{c*}	15.81±0.62^{A*}

Mean ± standard deviation of three replicates. Different lowercase letters within a row indicate significant differences ($p \leq 0.05$) among phenolic content of non-digested and digested samples. Different uppercase letters within a row indicate significant differences ($p \leq 0.05$) between bioaccessibility values at each digestion step. The asterisk indicates significant differences ($p \leq 0.05$) for phenolic content and bioaccessibility between M and SM at each digestion step.

GD: gastric digestion; GID: gastrointestinal digestion; nd: not detected; dp-3-sph-5-rhm: delphinidin-3-sophoroside-5-rhamnoside; dp-3-glc-5-rhm: delphinidin-3-glucoside-5 rhamnoside; dp-3-glc-5-acrhm: delphinidin-3-acetylramnoside; glcat d: galocatechin derivative; Ca 4-O-glc: caffeic acid 4-O-glucoside; glcat: galocatechin; rut: rutin; myr-3-rut: myricetin-3-rutinoside; pro dimer: procyanidin dimer; M: mashua; SM: steamed mashua; TIPC: Total individual phenolic content

¹ Values are percentage with respect to TIPC recovered after each digestion phase

² Sum of non-acylated anthocyanins

³ Sum of acylated anthocyanins

⁴ Sum of galocatechin derivatives

⁵ Sum of caffeic acid 4-*O*-glucosides

Table 3. Antioxidant, α -amylase and α -glucosidase inhibitory activities of black maca powder before and after gastric and gastroduodenal digestion*

	Non-digested	GD	GID
ORAC ($\mu\text{mol TE/g dw}$)	73.81 $\pm$ 7.00 ^c	172.68 $\pm$ 13.75 ^a	142.94 $\pm$ 10.84 ^b
α -amylase inhibition (IC_{50} , mg/mL)	3.28 $\pm$ 0.52 ^b	3.84 $\pm$ 0.34 ^b	9.88 $\pm$ 3.26 ^a
α -glucosidase inhibition (IC_{50} , mg/mL)			
Sucrase activity	2.77 $\pm$ 0.24 ^b	3.45 $\pm$ 0.28 ^b	5.25 $\pm$ 1.66 ^a
Maltase activity	3.56 $\pm$ 0.35	nd	nd

*Mean $\pm$ standard deviation of three replicates. Different letters within a row indicate significant differences ($p \leq 0.05$) for each studied parameter among samples. dw: dry weight; GD: gastric digestion; GID: gastrointestinal digestion; IC_{50} : inhibitory concentration that reduces 50% the original enzymatic activity; nd: not detected; ORAC: oxygen radical absorbance capacity; TE: Trolox equivalents.

Table 4. Antioxidant, α -amylase and α -glucosidase inhibitory activities of black mashua and steamed mashua powders before and after gastric and gastroduodenal digestion*

	M			SM		
	Non-digested	GD	GID	Non-digested	GD	GID
ORAC ($\mu\text{mol TE/g dw}$)	158.54 $\pm$ 13.18 ^c	607.26 $\pm$ 17.60 ^a	446.68 $\pm$ 39.54 ^b	187.30 $\pm$ 15.82 ^{c*}	491.40 $\pm$ 41.04 ^{a*}	279.23 $\pm$ 46.25 ^{b*}
α -amylase inhibition (IC_{50} , mg/mL)	2.09 $\pm$ 0.16 ^b	2.94 $\pm$ 0.08 ^a	nd	5.69 $\pm$ 0.16 ^{b*}	10.51 $\pm$ 1.84 ^{a*}	nd
α -glucosidase inhibition (IC_{50} , mg/mL)						
Sucrase activity	0.83 $\pm$ 0.03 ^c	0.91 $\pm$ 0.01 ^b	1.58 $\pm$ 0.02 ^a	3.66 $\pm$ 0.17 ^{b*}	5.03 $\pm$ 0.29 ^{a*}	5.86 $\pm$ 0.54 ^{a*}
Maltase activity	1.22 $\pm$ 0.05 ^c	2.85 $\pm$ 0.25 ^b	5.02 $\pm$ 0.25 ^a	2.93 $\pm$ 0.27 [*]	nd	nd

*Mean $\pm$ standard deviation (SD) of three replicates. The different letters within a row indicate significant differences ($p \leq 0.05$) for each studied parameter among non-digested and digested samples. The asterisk indicates significant differences ($p \leq 0.05$) for each studied parameter between M and SM at each digestion step. dw: dry weight; GD: gastric digestion; GID: gastrointestinal digestion; IC_{50} : inhibitory concentration that reduces 50% the original enzymatic activity; nd: not detected; M: mashua; ORAC: oxygen radical absorbance capacity; SM: steamed mashua. TE: Trolox equivalents.

Figure 1. Morphological features of black maca (A) and mashua (B) tubers.

Highlights

- Abundance of phenolic acids and anthocyanins in maca and mashua, respectively
- Higher phenolic content and antioxidant activity observed during digestion of mashua
- Mashua phenolics showed higher inhibition of carbohydrate hydrolysis during digestion
- Steaming reduces bioaccessibility of mashua phenolics and inhibition of glycosidases

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: