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Development and validation of a liquid chromatographic method to quantify sucrose, glucose, and fructose in tubers of *Solanum tuberosum* Group Phureja

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ABSTRACT

A High Performance Liquid Chromatography (HPLC) method was developed and validated to quantify sucrose (non-reducing sugar), glucose, and fructose (reducing sugars) in raw tubers of *Solanum tuberosum* Group Phureja. Chromatographic analysis was performed using an AMINEX HPX 87H column, at 18 °C, linked to a refraction index detector, at 35 °C. The eluent was 10 mM sulfuric acid. The conditions established for the method provided an optimum separation of sugars, citric acid, and malic acid, with resolution values higher or equal to one. Among the four sugar extraction methods tested, the double 50% (v/v) aqueous methanol extraction gave the highest level of analytes. Recovery of this extraction method ranged between 94.14 and 99.77%. The HPLC method was validated for repeatability, reproducibility, linearity, and limits of detection, and quantification. Relative standard deviation was found to be lower than five, when testing repeatability and reproducibility, which is suitable considering a range of acceptability from 5.3 to 7.3. Additionally, the regression analyses supported the method linearity in a range of quantification from 3 to 100 mg/L with regression coefficients values greater than 0.998 for the three analytes. Limits of detection were 3.0 mg/L for the three sugars and limits of quantification were 2.0 mg/L for sucrose and 3.0 mg/L for glucose and fructose. Four Colombian commercial cultivars (Criolla Guaneña, Criolla Paisa, Criolla Galeras, and Criolla Colombia) and five landrace accessions from the Colombian Core Collection of Group Phureja were grown in the district of Usme (Bogotá) fields to analyze their sugar contents. Sucrose, glucose, and fructose contents were found ranging from 0.93 to 3.11 g/100 g tuber dried weight (DW), from 0.25 to 4.53 g/100 g tuber DW, and from 0.10 to 1.49 g/100 g tuber DW, respectively. Therefore, a high range in the variability of sugar contents was found among genotypes. However, the variability was low among technical replicates of the same genotype, revealing an accurate quantification of sugars in Group Phureja. This method can be used to assess the amount of reducing and non-reducing sugars accumulation in potato germplasm.

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1. Introduction

Potato (*Solanum tuberosum*) plays an important role in worldwide food security and nutrition as it is the third crop of global relevance [1]. Tetraploid potatoes are the most cultivated worldwide, however, there is an interesting potential for the widespread use of diploid genotypes, 'Creole potato', that are known for their round tubers with yellow flesh and skin, and outstanding organoleptic properties [2]. Creole potato genotypes belong to the cultivated *S. tuberosum* Group Phureja, consisting primarily on diploid potatoes, that present a center of diversity in the south Andean region of Colombia and the north of Ecuador [3]. This cultivated group is characterized by lack of tuber dormancy, short day growing period, and short vegetative season (120 days) [4]. Colombia is positioned as the greatest producer, consumer, and exporter of these genotypes [5]. As owners of a high diversity of Phureja potatoes, it is crucial to generate knowledge on the processing quality, especially the sugar type and contents.

Glucose and fructose (reducing sugars) accumulation is a phenomenon that increases at low temperatures, as these compounds

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contribute to freezing tolerance [6]. The plant uses sucrose (non-reducing sugar) as the main source for the accumulation of reducing sugars in the tuber. Non-enzymatic browning is the result of the reaction between free amino acids and reducing sugars at high temperatures during frying process, generating the production of dark pigments and rancid flavors in chips or French fries, due to the Maillard reaction [7]. Reducing sugars contents are conditioned by the levels of starch synthesis, degradation, and transport, which depend on the genotype and the temperatures at which the tubers are stored [7,8].

A creole potato cultivar for frying has yet not been developed and the current cultivars show several undesirable properties for processing, mainly because of the accumulation of high levels of reducing sugars in tubers leading to non-enzymatic browning [9]. Due to a worldwide increase in the demand of processed food, the potato processing industry is a fast growing sector [5]. Hence, Colombia has a big challenge in the development of Phureja cultivars for frying. The phenotypic assessment of potato frying quality has been performed frequently by means of visual scales of frying color [9–11]. There is no report on the concentration of sucrose, glucose, and fructose of Phureja germplasm collections. Such quantitative data are important for the breeding programs, to implement strategies such as association mapping where precise quantification of the phenotype is imperative for the genetic association analyses [12].

Chromatographic methods are the most powerful analytical techniques for the identification and quantification of monosaccharides and oligosaccharides in foods. High Performance Liquid Chromatography (HPLC) is currently the most common chromatographic method for analyzing these compounds as it is capable of rapid, specific, sensitive, and precise measurements [13]. For method validation it is necessary to demonstrate that a particular protocol applied to a sample is suitable for obtaining analytical results with an acceptable uncertainty level [14], including the protocol for the extraction of the analytes of interest.

Sugar extraction methods for potato tubers include the use of e.g. 80% (v/v) aqueous ethanol either at 80 °C [15] or at 60 °C [16], 50% (v/v) aqueous methanol [17], 100% (v/v) methanol with activated charcoal at room temperature [18], or water [19]. Ion exchange resin columns are of widespread use in carbohydrate HPLC analysis since they do not require complex eluents for effective separations [20,21]. Extracts of sugars from potato tubers have been analyzed using these columns, e.g. with a Carbopac PA1 column using gradient elution with sodium hydroxide [17,19], an Inertsil NH_2 column with 80% (v/v) aqueous acetonitrile as eluent [22], and with an AMINEX HPX 87H column using sulfuric acid 8 mM for elution [23]. In the mentioned studies, nevertheless, there are neither details on method development nor in validation steps for sugar extraction and chromatographic analysis. This study, therefore, reports the development and validation of an extraction method and a HPLC method for the identification and quantification of sucrose, glucose, and fructose in *S. tuberosum* Group Phureja raw tubers, involving the method implementation using an AMINEX HPX 87H ion exchange column in the analysis of nine genotypes.

2. Materials and methods

2.1. Chemicals

Sulfuric acid (98% analytical grade) and methanol (99.96% analytical grade) were from J.T. Baker (Center Valley, PA, USA). Sucrose, glucose, and fructose HPLC standards were provided by Sigma–Aldrich (St. Louis, MO, USA). Water was obtained from a Water ProPs purification system (Labconco, Kansas, MO, USA).

2.2. Plant material and field design

Potato tubers were produced in the field in a loam soil (pH 5.02), in the district of Usme (Bogotá, Colombia; with an altitude of 3400 m above sea level, latitude of 4°20′23″N and longitude of 74°10′55″W). Tubers were sown on the beginning of April 2013 and plants were harvested on the mid of August 2013. Tuber maturity was assessed at the end of the growth season (140–150 days in these altitudes), when foliage was senescent and the skin was set to the tubers [24,25]. A randomized block design with three replicates was used to study the sugar content of four Colombian commercial cultivars (Criolla Guaneña, Criolla Paisa, Criolla Galeras, and Criolla Colombia) and five landraces from the Colombian Core Collection of Group Phureja (CCC) (CCC 8, CCC 52, CCC 80, CCC 108, and CCC 123). Each experimental unit consisted of tubers from one to three plants from each genotype in each block. Tubers from the three blocks were bulked to generate a composite sample of each genotype. Then, sugar content analyses were done with three technical replicates.

2.3. Tuber sample preparation

After harvest, mature and healthy tubers without mechanical damages were washed with distilled water and stored at 18 °C. Two days later, the tubers were cut into slices (0.3–0.5 cm) and stored at −20 °C. The frozen slices were freeze-dried for 72 h using a freeze drier model Free Zone 7806020 (Labconco, Kansas, MO, USA), homogenized using a domestic blender, and fine ground using a pestle and a mortar. Homogenized tissue was sieved with a mesh, obtaining a particle size of maximum 0.8 mm.

2.4. Optimization of sugar extraction and chromatographic analysis

2.4.1. Optimization of sugar extraction

Four extraction methods were tested using 0.5 g of freeze-dried tubers of Criolla Galeras: (i) extraction with 4 mL HPLC grade water at 92 °C [26]; (ii) extraction with 4 mL 50% (v/v) aqueous methanol using reflux [27]; (iii) extraction with 4 mL aqueous methanol in activated charcoal [18]; (iv) double extraction with 4 mL aqueous methanol [28]. Each of these four extraction methods were repeated three times, thus three technical replicates were performed. Methanol was removed from the extracts by rotoevaporation and diluted to 10 mL in a volumetric flask with 10 mM sulfuric acid. Extracts were purified using 500 mg of C-18 cartridges (Agilent Technologies, Santa Clara, CA, USA), to remove less polar compounds and avoid possible co-elution with sugars during HPLC analysis [29]. Cartridges were activated with 2 mL of methanol and washed with 3 mL of 10 mM sulfuric acid. Then, 1 mL of extract was loaded into the cartridge and washed with 3 mL of 10 mM sulfuric acid. The aqueous eluate was taken to a 10 mL volumetric flask and topped up with 10 mM sulfuric acid.

Recovery of the selected method was calculated in extractions of raw tissue from Criolla Guaneña, by analyzing triplicate samples spiked with a standard mixture of 10 mg/L and three un-spiked samples, according to Eq. (1).

$$\%\,\text{Recovery} = \frac{C_{spk} - C_s}{C_{ad}} \times 100 \quad (1)$$

where C_{spk} is the average concentration of spiked samples, C_s is the average concentration of sugars from the original sample, and C_{ad} is the concentration of standards added to spiked samples.

2.4.2. Optimization of chromatographic analysis

The samples were analyzed in an UHPLC Ultimate 3000 (Dionex, Sunnyvale, CA, USA) equipped with a pump, an autosampler and

a refraction index (RI) detector (Shodex, New York, NY, USA), at 35 °C, using an AMINEX HPX 87H column (300 mm length × 7.8 mm particle size) (Biorad, Hercules, CA, USA), with a pre-column (30 mm × 4.6 mm). Column temperature and concentration of sulfuric acid were tested in an interval from 18 to 35 °C and from 5 to 12 mM sulfuric acid. Those conditions that yielded the best resolution in compound separation were selected for sugar content analysis. The elution was isocratic during 35 min. A volume of 20 μL was injected per sample. The compound identification was based on comparison of retention time and co-elution after spiking with authentic standards. Quantification was performed by the external standard method. Calibration curves were established using a mixture of sucrose, glucose, and fructose standards, at concentrations ranging from 5 to 100 mg/L. Operation of the instrument and data processing were implemented using Chromeleon v. 7.1.2. (Dionex, Sunnyvale, CA, USA).

Area of the peaks was integrated according to the maximum peak width from the baseline. Compound separation was determined based on the resolution for each pair of peaks, in injections of three mixtures of standards, at a concentration of 100 mg/L, using the European Pharmacopeia formula shown in Eq. (2).

$$\text{Resolution} = \left| \frac{t_{\text{RefPeak}} - t_{\text{R}}}{W_{50\%,\text{RefPeak}} + W_{50\%,\text{R}}} \right| \quad (2)$$

where t_R is the retention time of the current peak, $t_{RefPeak}$ is the retention time of the reference peak which by default is the peak after the current peak, and $W_{50\%,R}$ and $W_{50\%,RefPeak}$ are the width of these two peaks at 50% of the peak height.

2.5. Chromatographic method validation and analysis of sugar content in a set of Group Phureja genotypes

The method developed above was validated for repeatability, reproducibility, linearity, and limits of detection (LOD), and quantification (LOQ). Inter-day repeatability was assessed over three days using a different 40 mg/L mixture of standards. Similarly, the reproducibility was evaluated based on five injections in the same day of a 40 mg/L mixture of standards. The Relative Standard Deviation (RSD) was used to evaluate the repeatability and reproducibility. AOAC's Peer Verified Method Program (PVMP) levels of acceptability of RSD values for given concentrations of the analyte, were used as a reference to analyze the precision of the method [30].

Linearity was evaluated based on linear regression analyses of three calibration curves run in different days, at concentrations of 5, 10, 20, 40, 60, 80, and 100 mg/L. The peak areas for the same concentration were averaged. Using this data a linear regression model was developed, and a regression coefficient greater than 0.998 was considered to have a linear relationship between the peak area and the concentration of the analyte [31,32]. LOD and LOQ were established using a mixture of standards with concentrations of 0.5, 1, 2, 3, 4, and 5 mg/L, prepared and injected in triplicates. LOD was visually assessed by identifying the minimum concentration at which each analyte was reliably detected in the three injections. Likewise, LOQ was determined based on the minimum concentration of analyte to which the peak area response and concentration showed linearity [32]. Thus, a regression model was settled using the average areas of the injections with lower concentrations. Using this validated method, three technical replicates, for each freeze-dried tuber sample, of nine genotypes were analyzed.

2.6. Statistical analyses

First, an analysis of variance of a completely randomized design was carried out to determine if at least one sugar extraction method was different from the others. Then, the Tukey's test was employed to identify differences among mean values, to select the extraction method that gave the highest level of extraction. Regression analyses for linearity and LOQ included testing the hypothesis for the model significance using the F statistic and testing the significance of the model's slope using a two-tailed t test. Hypotheses were tested with a level of significance of $p < 0.05$. All the analyses were carried out using R software v. 3.1. [33].

3. Results and discussion

3.1. Optimization of sugar extraction and chromatographic analysis

3.1.1. Optimization of sugar extraction

The analysis of variance revealed that for each sugar content at least one sugar extraction methodology was significantly different from the others. Table 1 shows the average contents of sucrose, glucose, and fructose for each method. Double extraction with 50% (v/v) aqueous methanol yielded the highest extraction levels for each analyte as well as it was determined that the mean value for this extraction method was significantly different from the others in glucose and fructose contents according to the Tukey's test. Despite sucrose mean value in this method was not significantly different from reflux with 50% (v/v) aqueous methanol, it is important to underline that the mean value for the other two sugars was significantly different, and that double extraction method allows carrying out an easier parallel processing of more samples than reflux.

Karkacier et al. [28] compared the sugar contents of apple samples extracted using water and the methanol extraction method adapted herein. Contrary to our results, these authors concluded that water was a more effective solvent than methanol for sugar extraction, since in the latter not all the sugars were soluble, because of solvent vaporization. In contrast, Johansen et al. [34] concluded that water and 50% alcohol (methanol or ethanol) produced similar sugar extraction levels and that during water extraction there was the risk of starch and oligosaccharides degradation due to enzymatic action.

Whether enzymatic degradation of starch occurred during water extraction, this phenomenon was overcome by the higher extraction power of 50% (v/v) aqueous methanol, since in all cases methanol extractions yielded higher sugar contents than those with water extraction. This result can be explained because the method used with water was with a temperature (92 °C) that caused tuber protein denaturation, which later might have trapped soluble sugars and diminished their extractions [35]. From

Table 1
Average concentrations of sugars for each extraction method. Averages with different letters in each column indicate significant differences according to Tukey's test ($p < 0.05$). Bold values show the highest contents of each analyte.

Method	Average sucrose concentration (mg/L)	Average glucose concentration (mg/L)	Average fructose concentration (mg/L)
Water	2566.22 ± 81.29 a	308.2 ± 3.14 a	747.44 ± 3.36 a
Methanol double treatment	**4925.64 ± 240.2 b**	**1035.36 ± 50.74 b**	**1487.26 ± 81.24 b**
Methanol with activated charcoal	3479.68 ± 189.74 c	672.42 ± 60.28 c	939.16 ± 82.79 ac
Methanol with reflux	4322.76 ± 417.87 b	817.85 ± 91.58 c	1169.59 ± 135.31 c

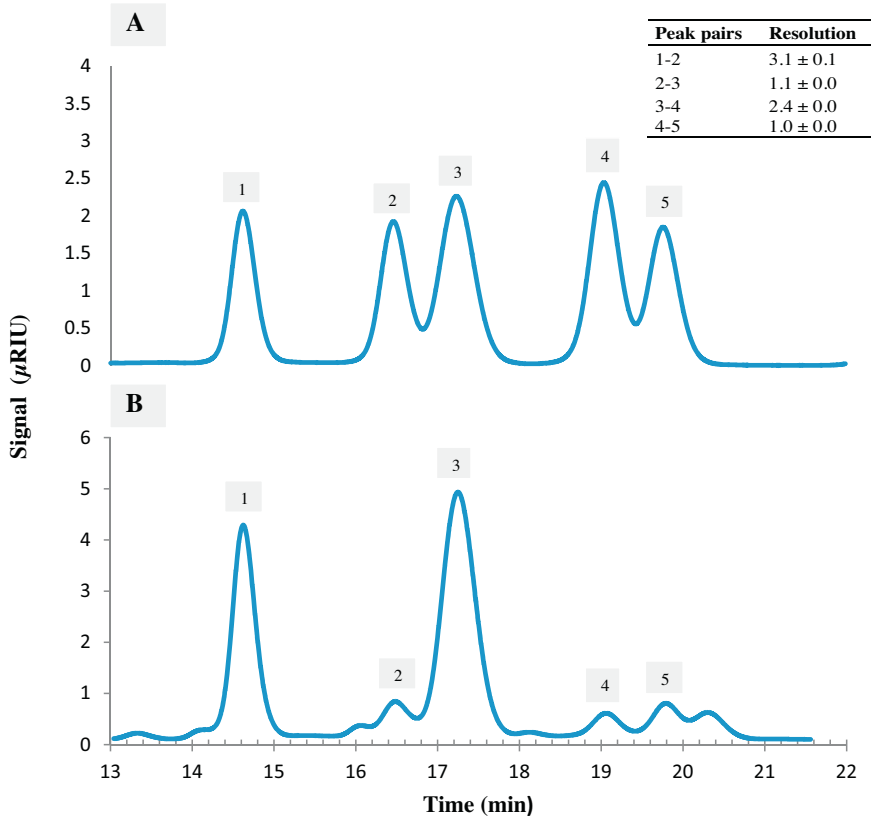

Fig. 1. HPLC-chromatograms illustrating (1) sucrose, (2) citric acid, (3) glucose, (4) fructose and (5) malic acid. (A) Mixture of sugars and organic acid standards in a concentration of 100 mg/L. Insert shows resolution for each pair of peaks from chromatogram (e.g. resolution for peaks 1–2 means resolution for sucrose and citric acid). (B) Sample of *S. uberosum* Group Phureja accession CCC 52. Concentrations found in accession were as follows: (1) 153.90 mg/L; (3) 225.32 mg/L; (4) 21.72 mg/L. μRIU: micro refractive index units.

the methods that use methanol, the one using activated charcoal yielded less sugar contents suggesting that charcoal could have adsorbed sugars into its carbon surface due to its preference towards organic molecules [36].

Thus, recovery analysis was performed with the double extraction method, revealing the highest percentages for sucrose (99.77 ± 1.98) and glucose (99.69 ± 1.55). The lowest recovery was found for fructose (94.14 ± 1.00), indicating that this compound was more prone to be lost during sample processing. This analysis demonstrates that extraction and sample purification interfere somehow in the loss of sugar contents during those procedures. Fructose data presented were not adjusted by its recovery value. Recovery values presented were within the acceptability range (90–107%, w/v) for analytes at a concentration of 100 mg/L [14].

3.1.2. Optimization of chromatographic analysis

From the different concentration values of sulfuric acid and temperatures of the column that were tested, a concentration of 10 mM sulfuric acid, at a flow rate of 0.3 mL/min, and a column temperature of 18 °C were the conditions that resulted in the best resolution for the compounds analyzed. The conditions established for chromatographic analysis allowed not only the separation of sucrose, glucose, and fructose, but also the separation and identification of two additional peaks corresponding to citric acid and malic acid (Fig. 1A). Selectivity of the method was assessed by checking the UV–vis answer of each chromatographic peak. The absence of UV–vis response in the peaks corresponding to the sugars studied was an indicative of the absence of compounds such as organic acids or phenolic compounds co-eluting with the sugars, compounds that are expected to be present in potato tuber extracts [37].

Resolution for each pair of the identified peaks is shown in Fig. 1A, using a mixture of sugars and organic acid standards in a concentration of 100 mg/L. Less resolved peaks were those from citric acid/glucose and fructose/malic acid with resolution values close to one. Authors as Kupiec [38] have reported that resolution values equal or greater than one indicate appropriate quality in compound separation. Fig. 1B shows a chromatogram of a potato extract from accession CCC 52 with sucrose, glucose, and fructose quantifications revealing as well an adequate separation of the compounds analyzed. Thus, the chromatographic method established provides good separation as well as proper quantification of sugars, supported by a satisfactory measurement of glucose and fructose containing citric acid and malic acid in the mixture of standards and in the sample.

Citric acid and malic acid play important roles in the Krebs cycle and are the most abundant organic acids in potato tubers [39,40]. It was expected, therefore to detect these compounds in Phureja tubers eluting close to sugars using an AMINEX 87H column [21]. Authors do not agree in specific malic acid and citric acid roles and accumulation trends in the physiological processes during storage [39,41,42]. Even though, it is reported that both acids increases their amounts when the tubers are stored at low temperatures [41]. Consequently, for the better understanding of the dynamics of sugars and major organic acids during storage in diploid potatoes, it might be appropriate to use this chromatographic method, as it is possible to simultaneously quantify sugars and major potato organic acids using the RI detector. It is important to underline, however, that UV detection is more sensitive than RI detection for the quantification of organic acids. In addition, the simultaneous quantification of sugars and organic acids are useful in the characterization of food

Table 2
Reproducibility and repeatability analysis of each analyte using a 40 mg/L mixture of standards.

Sugar	Reproducibility		Repeatability	
	Average (mg/L)	RSD	Average (mg/L)	RSD
Sucrose	40.08 ± 0.49	1.21	42.06 ± 2.40	5.71
Glucose	40.42 ± 0.34	0.83	41.33 ± 1.52	3.67
Fructose	40.41 ± 0.29	0.72	40.49 ± 1.80	4.44

RSD, relative standard deviation.

products as wine because these contents analyses are required for quality evaluation [43].

Using the same type of column to quantify sugars in the Colombian tetraploid cultivar R-12 (Diacol Capiro), Fonseca-Sainea and Urueña-Avila [23] did not report the presence of additional peaks corresponding to organic acids. This fact can be explained because of lower concentration of sulfuric acid (8 mM) and higher column temperature (35 °C) used by them, did not allow resolving acids from sugars or due to the existence of non-detectable amounts of citric acid and malic acid in the cultivar studied. In contrast, Eyéghé-Bickong et al. [44] reported the co-elution of fructose and malic acid, using AMINEX 87H column, operated at 55 °C with 5 mM sulfuric acid in wine and grapevine samples. The chromatographic method used in our research shows the possibility to resolve fructose from malic acid without using organic modifiers in sulfuric acid solutions as proposed by Castellari et al. [43], but by increasing acid concentration and diminishing column temperature. In this scenario, it is expected an accurate quantification of fructose with RI detection as it separates from malic acid in the RI detection. This approach is more suitable than those that comprise the quantification by RI detection of sugars and UV detection of organic acids, without resolving organic acids from sugars in RI detection [45,46].

3.2. Chromatographic method validation and analysis of sugar content in a set of Group Phureja genotypes

3.2.1. Method validation

Reproducibility and repeatability were determined for each analyte according to the RSD values shown in Table 2. As injections were performed on three different days, the repeatability assay revealed a higher RSD values than reproducibility. Taverniers et al. [14] indicated that the AOAC's PVMP proposes levels of acceptability of RSD values with greater accuracy than the Horwitz function [47]. Hence, for an analyte in a concentration ranging from 10 to 100 mg/L, a RSD between 5.3 and 7.3 is acceptable. Mixtures of 40 mg/L standards were used for both assays, yielding RSD values even lower than the range previously mentioned. The RSD values obtained when testing reproducibility and repeatability, assures the precision of the method developed for the quantification of sucrose, glucose, and fructose.

The averaged regression coefficients for the independent calibration curves analyzed revealed values greater than 0.998 (Table 1 of Supplementary Data), indicating an acceptable fit of the data to the regression curves which supports a proportional relationship between the response of the analyte and its concentration [31]. Using the averaged values of areas in different concentrations, a regression model was fitted for each sugar. The regression coefficients obtained revealed an acceptable fit of the averaged data for all sugars; as well, the *F* tests for the regression showed significance ($p < 0.05$). The two-tailed *t* test for the slope ($p < 0.05$) demonstrated that the values were different from zero which indicated that the sugar concentration had a significant effect on peak area (Table 1 of Supplementary Data). These analyses supports the significance of the adjusted models, which reinforces the linearity of the analyte response in the range studied and the accuracy in sample quantification performed at different times with different calibration curves.

LOD and LOQ showed the same values for both parameters in glucose and fructose (3 mg/L) and a lower value for sucrose in LOD (2 mg/L). The adjusted models for LOQ analysis and their slopes were significant according to the *t* and *F* tests respectively, indicating that the method quantification was linear at concentrations ranging from 3 to 100 mg/L for the three sugars (Table 2 of Supplementary Data). To summarize, the validation analyses performed demonstrated the method precision in a range of linearity from 3 to 100 mg/L.

3.2.2. Sugar content in Group Phureja genotypes based on validated method

The sugar contents of nine Phureja genotypes were calculated and shown in Table 3. According to the AOAC's levels of acceptability of RSD (5.3–7.3), two values were out of range, but considering the Horwitz function these values are acceptable [14,47]. Besides, these RSD values belong to glucose and fructose, and the quantification of these compounds showed greater variability which was reinforced by higher RSD means and higher RSD deviations with respect to sucrose values. Thus, the reliability found in the quantification of these genotypes supported the method validation presented here.

Glucose and fructose contents found in Phureja potatoes were not equimolar as fructose amounts were lower. This result agrees with a potential high activity of fructokinase which is responsible for fructose metabolism into hexose–phosphate cycle, thus diminishing the fructose content in tubers [48]. There was a high

Table 3
Average content of sugars in tubers of commercial cultivars and landraces from CCC. Bold numbers indicate those RSD values out of the levels of acceptability established by the AOAC [14,30].

Genotype	Sucrose average		Glucose average		Fructose average		Reducing sugars	Total sugars
	(g/100 g tuber DW)	RSD	(g/100 g tuber DW)	RSD	(g/100 g tuber DW)	RSD	(g/100 g tuber DW)	(g/100 g tuber DW)
Guaneña	1.47 ± 0.04	3.02	0.27 ± 0.02	**8.13**	0.10 ± 0.01	4.88	0.36 ± 0.04	1.83 ± 0.08
Paisa	2.10 ± 0.05	2.24	0.26 ± 0.01	4.89	0.13 ± 0.01	4.61	0.39 ± 0.01	2.49 ± 0.04
Galeras	0.93 ± 0.05	5.74	0.29 ± 0.02	5.98	0.12 ± 0.01	**8.50**	0.40 ± 0.03	1.33 ± 0.08
Colombia	1.31 ± 0.04	2.76	0.25 ± 0.01	2.53	0.15 ± 0.01	5.05	0.40 ± 0.01	1.71 ± 0.05
CCC 8	1.05 ± 0.05	4.65	0.70 ± 0.04	5.60	0.32 ± 0.01	3.33	1.00 ± 0.07	2.05 ± 0.11
CCC 52	3.11 ± 0.04	1.16	4.53 ± 0.03	1.06	0.44 ± 0.01	2.17	4.97 ± 0.04	8.09 ± 0.07
CCC 80	1.36 ± 0.05	3.52	0.44 ± 0.02	4.36	0.14 ± 0.01	5.38	0.59 ± 0.02	1.94 ± 0.07
CCC 108	1.92 ± 0.04	1.98	2.79 ± 0.08	2.74	1.49 ± 0.02	1.10	4.28 ± 0.09	6.19 ± 0.13
CCC 123	1.99 ± 0.05	2.73	1.50 ± 0.06	4.17	0.35 ± 0.01	2.62	1.89 ± 0.02	3.85 ± 0.01
Average	1.46 ± 0.44	3.09 ± 1.40	1.23 ± 1.50	4.38 ± 2.11	0.36 ± 0.44	4.18 ± 2.18	1.59 ± 1.80	3.28 ± 2.35
Maximum	3.11	5.74	4.53	8.13	1.48	8.50	4.97	8.09
Minimum	0.93	1.16	0.25	1.06	0.10	1.10	0.36	1.33

CCC, Colombian Core Collection of *Solanum tuberosum* Group Phureja; DW, dried weight; RSD, relative standard deviation.

variability in the sugar content among the four commercial and five CCC landraces studied (e.g. sucrose content ranged from 0.93 to 3.11 g/100 g tuber DW). McCann et al. [16] analyzed, based on HPLC, the sugar content of two Group Phureja accessions stored at 2 °C during three months. As these authors studied tubers from plants grown from botanical seeds of these accessions subjected to cold storage, wider ranges for sugar contents were found, especially for sucrose (ranging from 1.6 to 16.9 g/100 g tuber DW). A detailed multi-environmental study of sugar contents of all genotypes from CCC using this HPLC method is necessary to conclude about the current extent of the natural variation of sugar contents in Colombian Phureja germplasm.

4. Conclusions

The chromatographic method developed and validated allows a simple and appropriate quantification of sucrose, glucose, and fructose in *S. tuberosum* Group Phureja, also offering the possibility of their simultaneous quantification with the most abundant organic acids (citric acid and malic acid) in potato using a RI detector. In addition, in comparison with water extraction and 50% (v/v) aqueous methanol extractions with reflux and activated charcoal, the double extraction method with 50% (v/v) aqueous methanol provides higher sugar contents, an appropriate recovery, and it is easy to implement when having a large number of samples as required for the assessment of germplasm collections. With the purpose of a wider knowledge of the natural variation of sugar contents in Group Phureja, it is necessary to include all genotypes from CCC. Therefore, the chromatographic method established will contribute to an accurate phenotypic characterization of this collection that will impact in the understanding of the process of reducing and non-reducing sugars accumulation in tubers of Phureja potatoes from Colombia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2014.10.039.

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