**Cooking dependent loss of metabolites in potato breeding lines and their wild and landrace relatives**

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# Abstract

Elucidating and improving the nutritional value of staple crops is an important focus in breeding programs. Hence, wild and cultivated potatoes have been metabolite profiled to assess metabolic plasticity present in potato tubers and changes that occur in these after cooking. The present data highlighted metabolic similarities of certain wild species to breeding lines, indicating preference during domestication. In the case of potato, the cooking process has to be taken into consideration for the assessment of nutritional quality. The degree of nutritional change during cooking depends on several factors including cooking method, stability of metabolites with nutritional properties and chemotype of the varietal matrix. The differences between raw and boiled tubers were similar within each germplasm group. No notable distinctions of total phenolic levels could be observed within germplasm groups, whereas total carotenoids and glycoalkaloids were significantly decreased. Genotypes/cultivars with notable changes upon cooking distinguished them within their genotype group.

**Keywords**: potato, metabolite profiling, phenolic compounds, nutrients, cooking, wild potatoes

**Abbreviations:** GC-MS, gas chromatography mass spectrometry; IPP, isopentenyl diphosphate derived metabolites; LC-MS, liquid chromatography mass spectrometry; PCA, principle component analysis; UPLC, ultra-performance liquid chromatography; CIP, International Potato Center; W, wild potatoes; N, native landraces/cultivars; NM, bred native hybrids; V, advanced breeding lines

# Introduction

Potato breeding programs have typically focused on yield, appearance and processing quality of tuber and biotic/ abiotic stress tolerance as priority traits (Bradshaw et al., 1994; Tarn et al., 2010). However, important criteria for consumers are flavour and more recently nutritional quality in potato. To date, potato and its products remains one of the most consumed staple food crops worldwide (McGregor, 2007). Two compound groups responsible for undesired bitter flavour are glycoalkaloids and phenolics. Glycoalkaloid and phenolic content in potato is variety dependent and can accumulate in tubers due to environmental stress, such as high temperatures or drought (Benavides Manuel et al., 2017; Drapal et al., 2017). Phenolic compounds, despite their bitter taste, are ubiquitous antioxidants with many health promoting properties (Ezekiel et al., 2013).

Potato is naturally a good source of minerals, vitamins, antioxidants, protein and fibre (Zaheer et al., 2016). Most of these nutrients are assessed based on the recommended daily allowance criteria. In the case of protein, which comprises up to 10% of dry tuber weight, quality is determined by the quantity and proportion of different amino acids. Patatin is the main potato tuber protein and its quality scoring depends greatly on the genotype (Bártová et al., 2015). The improvement of nutritional properties of potato tubers can help reduce hidden hunger and improve human health in developing countries (Sosa et al., 2018). Hence, programs such as HarvestPlus support the breeding of biofortified potato varieties with increased micro-nutrients (e.g. iron, zinc) and antioxidants/vitamins (Andre et al., 2015; Sosa et al., 2018).

A key component of the breeding efforts in potato is the need to be the assessment of potato tuber properties after cooking. Thermal processing can have positive and negative effects on food components. It changes the digestibility of potatoes, which can influence the bioavailability of nutrients, but also affects the concentration of nutrients, depending on their chemical stability to temperature and pH changes (Tierno et al., 2015). Cooking methods for potato include boiling, baking and frying. Boiling was chosen in the present study, as it is the most readily accessible method for consumers and does not require the addition of fat, which interferes with technical measurements. Modern metabolomics is ideally placed to measure a large number of different compounds before and after cooking and assess the most suitable potato varieties in a diversity panel, which maintain quality after cooking.

Phylogenetic analysis of a wide range of potatoes including wild relatives, landraces and bred varieties indicates that cultivated potatoes have a monophyletic origin, arising from a group of extremely similar wild species classified in the *Solanum brevicaule* complex (Spooner et al., 2014). Comparing metabolic profiles for flavour and nutrient related compounds between these wild progenitors and domesticated potatoes may provide information on the role of flavour and tuber quality in potato domestication and insights for cultivar development. Hence, the aim of this study is to explore the range of biochemical compounds present in raw and cooked potato tubers from a panel of wild species, landraces and bred cultivars of *S. tuberosum* groups Tuberosum, Phureja, Goniocalyx and Stenotonum. A metabolite profiling approach was applied to determine differences among and within the germplasm groups and use the data to investigate cooking effects on biochemical compounds in different phenotypic backgrounds.

# Materials and Methods

## Plant material and experimental design

A panel of 32 advanced tetraploid potato breeding clones, 15 diploid landraces from Phureja and Stenotomum Groups, 15 diploid biofortified bred clones, and 10 wild genotypes from species indicated as ancestors of cultivated potato were grown in a field trial during 2016 in the highlands of Peru (Mullaca, Tayacaja, Huancavelica- 4,100 MASL) using local agronomic practices. The germplasm set was planted in four individual trials following each a randomized complete block design consisting of three blocks of 10-hill plots. Tubers of each clone were harvested at maturity by plot within the three blocks. For each genotype a number of 15 to 30 uniform-sized and visually health tubers were selected and used for: dry matter determination, amylose and amylopectin content and metabolite analysis. Two or four tubers per clone and replication were cut in quarters and two opposite quarters from either raw or boiled tubers were frozen in liquid nitrogen and freeze dried.

## Metabolite analysis

### Extraction of metabolites

Freeze dried tuber samples were ground to powder. Samples, including quality controls (pool of all samples, QC), were weighed (10.0 ± 0.5 mg) in plastic tubes and extracted as described previously (Nogueira et al., 2013). Aliquots of the polar and non-polar phase were immediately dried down after extraction. Analysis included duplicate extraction of two biological replicates, resulting in four replicates.

### Gas chromatography mass spectrometry (GC-MS) analysis

An aliquot of the polar phase (200 µl) was removed and internal standard (d4-Succinic acid, 10 µg) added before dry down. Dried samples were derivatised as previously described (Nogueira et al., 2013) and analysed by GC-MS based on literature (Enfissi et al., 2010), using a 10:1 split mode. Metabolites were identified with respect to an in-house library based on retention time, retention indices and mass spectrum (Nogueira et al., 2013) and quantified relatively to the internal standard.

### Liquid chromatography mass spectrometry (LC-MS) analysis

Each dried aliquot of the polar phase (700 µl) was resuspended in methanol/water (1:1, 100 µl) and internal standard (homogenistic acid, 1.25 µg) added. Samples (5 µl injection) were analysed by LC-ESI-QTof (Bruker Daltonics maXis, Bremen, Germany coupled to Dionex Softron UltiMate 3000, Gemering, Germany) with a solvent gradient 100% A (water and 0.1% formic acid) to 95% B (acetonitrile and 0.1% formic acid) at a flow rate of 0.2 ml/min on a reverse phase C18 column (250mm x 4.6mm; 5µm; YMC Inc., Hichrome) (Drapal et al., 2018). Data analysis was performed with R package metaMS (Shahaf et al., 2013; Wehrens et al., 2015) including an in-house library of analytical standards and a retention time window match of 0.5min (Drapal et al., 2018).

### Carotenoid analysis with ultrahigh performance liquid chromatography (UPLC)

Samples were weighed (50 ± 0.5 mg) and extracted with chloroform/methanol on ice as previously described (Nogueira et al., 2013). The pooled non-polar phase was dried, resuspended in ethyl acetate/acetonitrile (1:9) and analysed as previously described with UPLC coupled to a diode array detector (Nogueira et al., 2013). Carotenoids were identified by retention time, UV/visible light spectrum and comparison to analytical standards and quantified from dose-response curves (Fraser et al., 2000).

### Data processing and statistical analysis

The metabolites detected by each platform are listed in Table A.1 and A.2. Relative and absolute quantities from UPLC, LC-MS and GC-MS analysis were combined in Microsoft Excel (Microsoft Office, USA) and different statistical analysis performed as previously described (Drapal et al., 2018). Metabolite data was visualised as heat maps with MetaboAnalyst 4.0 (Chong et al., 2018). PCA analysis and hierarchical clustering was performed with Simca P 13.0.3.0 (Umetrics, Sweden). Differential expression with Benjamini-Yekutieli post-hoc correction, RV coefficient and Mantel test with Spearman correlation were performed with XLSTAT add-ins (Addinsoft, France).

# Results and discussion

## Metabolite diversity in potato tubers

For the present metabolite study a potato diversity panel was comprised from four different germplasm groups: advanced breeding lines of *S. tuberosum L. tuberosum* (V), wild diploid potatoes (W), traditional diploid cultivars/landraces of the *S. tuberosum L. Andigenum* group (N) and bred native hybrids for higher nutritional value (NM) (Table 1). The data analysis of the metabolite profiling focused on metabolites with phytochemical, nutritional and flavour properties. This led to the identification of 77 metabolites including amino acids, sugars, intermediates of the TCA cycle, phenolics, glycoalkaloids and carotenoids (Table A.1 and A.2).

The metabolite composition of raw and cooked potato tubers showed a significant correlation (*P*=0.0002) with a ~85% similarity between the two conditions. This has been previously reported for potato breeding lines and landraces (Tierno et al., 2015). The PCA plot showed that the majority of wild potatoes varied significantly in composition from native, bred hybrid and advanced potatoes (Fig. 1). The species *S. incamayoense* (W410) and *S. tacnaense* (W408) were grouped with the native accessions, supporting the hypothesis that these species are progenitors of the Andean landrace potatoes (Spooner et al., 2014). The diversity of chemotypes in wild potato reflects the high interspecific variability of several human health related metabolites identified among wild *Solanum* species (Aversano et al., 2017). The overlap of the native, bred hybrid and advanced clones coincides with genotyping studies and emphasises the selection of specific metabolic traits during domestication and breeding efforts (Hardigan et al., 2017; Spooner et al., 2007). The cluster patterns of domesticated/bred potatoes highlighted that genotypes can be identified by their chemotypes in both raw and cooked tubers (Ogura et al., 2016; Orgogozo et al., 2015). Furthermore, the metabolite analysis corroborates that the chemotypes of bred native hybrids was significantly changed as intended through the biofortification crossing program of CIP (Fig. 1). The bred native potatoes are hybrids of native cultivar groups Stenotomum, Goniocalyx and Phureja and had a total carotenoid content of 42 to 118 µg/g DW. This equates to 0.9 to 2.6 mg/100g FW (based on an average dry matter content ~22%), which was significantly higher than total carotenoid content measured in native cultivars in the present study and previously published (Brown et al., 2007; Burgos et al., 2013b).

## ****Hierarchical relationship between potato tubers based on metabolite profiles****

The chemotypic relationships between the varieties/genotypes within each germplasm group were further assessed based on the raw tuber material (Fig. 2). Interestingly, the grouping of wild potatoes and native landraces in the dendrogram seemed to reflect the species and cultivar level (Fig. 2B & D). Within the wild potatoes, the *S. candolleanum* genotypes (1 accession, W403 and W404) showed the closest intraspecies similarities, whereas the genotypes of *S. bukasovii* (2 accessions, W401, W409) and *S. multiinteruptum* (1 accession, W405 and W406) clustered within the same branch on different arms (Fig. 2B). The metabolite similarities of wild potatoes could be observed on a species but not on a taxonomic level as defined by Spooner et al. (2014). Based on this new classification *S. ambosinum* (W402)*,* and *S. bukasovii* are synonyms of taxon *S. candolleanum;* while *S. incamayonse* (W410) and *S. sparsipilum* (W407) are synonyms of taxon *S. brevicaule.* Neither of these two taxa showed close metabolic similarities between species within one taxon group. Additionally, a metabolic similarity to cultivated potatoes should be present between taxa *S. brevicaule* and *S. multiinterruptum*, as both are considered ancestors to the latter (Spooner et al., 2014). Only *S. incamayoense* (W410) showed this characteristic and clustered with the native/domesticated potatoes analysed in the present study (Fig. 1). The only other wild potato to cluster towards cultivated potatoes was *S. tacnaense* (W408).

The dendrogram of the *S. tuberosum L. Andigenum* native landraces (Fig 2D) showed a distinct separation along the cultivar groups Phureja, Goniocalyx and Stenotomum which are considered as a single species based on the current taxonomy (Ovchinnikova et al., 2011). The *S. stenotomum* landraces were grouped in both branches of the dendrogram, which coincides with the genotyping study published previously (Spooner et al., 2007). The tuber flesh colour of the native landraces varied from yellow (Phureja) to white/cream (Goniocalyx and Stenotomum). The metabolite data (Fig. 2D) emphasises that the observed tuber flesh colour (Table 1), based on carotenoid and phenolics content, plays a minor part in the overall metabolite composition of the tubers. Additionally, the data confirms the close relationship within the *S. tuberosum L. Andigenum* group which could be linked to shared ancestors as reviewed previously (Spooner et al., 2014).

The dendrograms of the advanced clones and the bred native hybrids did not show clear pedigree-based clustering (Fig. 2A & C). Full siblings such as the advanced clones V104 and V108 or V117 and V127 were found in different clusters. The same findings were apparent with the half-siblings V104, V105, V112 and V115 (Fig 2A). Similarly in the bred native hybrid group, full siblings NM208, NM209 and NM210 were found in different clusters (Fig 2C). This reflects the high heterozygosity that is a hallmark of outbred polyploid crops such as the cultivated potato (Sattler et al., 2016). The main chemotypic difference within the bred native hybrid group could be linked to four chemical classes (phenolics, intermediates of the TCA cycle, epoxy-carotenoids and glycoalkaloids). The levels of these metabolites were higher in accessions clustering in the top branch of the dendrogram (Fig. 2C). In a similar manner to that observed for the native landraces, the metabolite data of bred native hybrids showed that neither carotenoids nor anthocyanins –observed as the red/purple colouration of the tuber – are dominant factors in the metabolic composition of the tuber.

## Identification of metabolites specific to potato germplasm groups

For a more detailed analysis, the metabolite levels of raw and cooked tubers were averaged for each germplasm group (Fig. 3). The dendrogram of the heat map (Fig. 3) highlighted that the potato tubers analysed clustered primarily by germplasm group and secondarily by the processing state of the tubers. This suggests that the quantitative changes of metabolites during the cooking process are germplasm specific and correlate with the cultivation status of each group.

Wild potatoes were the most different (left branch Fig. 3) due to higher levels of glycoalkaloids (~7-fold) and phenolics (2-4-fold) compared to other potato groups. Furthermore, wild potatoes displayed a different quantitative profile of primary metabolites compared to the landraces/cultivated potatoes. This was partially expected as the cultivated potatoes were selected for e.g. their tuber size, starch quality, reduced glycoalkaloid levels and other metabolites related to consumer preferred flavour/taste traits (Hardigan et al., 2017). The second/right branch was separated into the bred native hybrids and a combined branch of the advanced breeding and native potatoes. The bred native hybrids showed higher levels of carotenoids (~1.3-fold) and organic acids (up to 2-fold), which was visually evident with a much more yellow tuber pigmentation than any of the other tubers analysed (Table 1). The main difference between the native landraces and the advanced clones was a higher level of amino acids (up to 3-fold) in the latter, with the exception of asparagine which was significantly higher in the wild species. Asparagine is the crucial participant in the production of acrylamide during cooking (Jung et al., 2006). Next to acrylamide, the Maillard reaction (non-enzymatic browning) produces other compounds influencing essential food quality attributes. The reduced levels of asparagine in the cultivated potatoes indicates that one of these attributes was not preferred during the domestication and breeding process of potatoes. Essential amino acids such as lysine, leucine, threonine, phenylalanine and valine levels were not significantly affected by cooking and were found in higher levels in the advanced clones and the bred native hybrids. These amino acids are probably linked with selectable traits in the breeding program of these lines. For example high chemical scores of phenylalanine and tyrosine were linked to the good quality of patatin proteins found in *S. goniocalyx* (Bártová et al., 2015). Additionally, tyrosine has been linked with the discolouration (enzymatic browning) of tubers and was decreased in potato tubers analysed, with the exception of two native and six advanced varieties (Corsini et al., 1992; Mondy et al., 1993). The lack of change in primary metabolism of the bred native hybrid lines compared to landraces and other cultivated potatoes, despite the high levels of carotenoids, is consistent with findings in sweet potato, where the carotenoid levels had no effect on the primary metabolism (Drapal et al., 2019). The native landraces showed quantitative changes of only a few single metabolites after cooking. This could present an interesting trait for breeding, if the raw metabolic composition meet consumer preferences.

## Metabolic changes in potatoes related to the cooking process

Overall, half of the metabolites identified were significantly increased or decreased between the raw and cooked tuber tissue (Table A.3), confirming that the cooking process affects a broad range of the metabolites present in potato tubers (Chaparro et al., 2018; Fabbri et al., 2016). A comparison of wild and cultivated potatoes can elucidate superior nutritional retention during cooking and potential cooking traits of wild potatoes for future breeding approaches.

The average values of all germplasm groups showed significantly decreased glycoalkaloid levels after cooking (Table 2). The wild potatoes had the least decrease of glycoalkaloids (~59%) during cooking. Glycoalkaloids cause bitter taste and are toxic to humans in high concentrations (Storey et al., 1992). Hence, the domestication process of potatoes has likely involved the selection of less bitter species/genotypes for cultivation as well as the development of processing methods that reduce the bitter taste (de Jonge et al., 2012). *S. incamayoense* (W410) is a distinct wild potatoes as it had a higher decrease (~83%) of glycoalkaloids compared to the average 59% decrease of other wild species. Other changes of *S. incamayoense* related to aroma/flavour such as a more intense decrease of amino acids, decreased phenolics levels and no change of ascorbic acid during cooking, indicates how this wild potato has a higher similarity to cultivated potatoes (Fig. 1) and why it would have been preferred during the domestication process (Spooner et al., 2014). Another interesting wild potato species was *S. candolleanum*, as its two genotypes (W403 and W404) had very similar metabolite profiles in raw tubers, but showed opposite trends for sugars and organic acids after the cooking process. Furthermore, one genotype (W403) had more pronounced changes of carotenoid and phenolic levels compared to the other genotype (W404) (Fig. 1). This may have implications for the selection of parents for breeding of potatoes with stable nutrient profiles.

Another finding from the averaged ratios was that the native potatoes had the lowest levels of free sugars in raw tubers (up to 50% less) and are the only group to show a significant increase of sugars (~67%) after cooking (Table 2). Both wild and native potatoes showed no correlation between free sugars before and after cooking, contrary to the positive correlation found in the cultivated bred native hybrids and advanced breeding lines (R=0.70 and 0.76, respectively; *P*<0.0001 for both; Fig. A.1). This indicates that the starch degradation and resulting release of sugars during cooking of starchy tubers are a regulated feature in the current breeding lines (van Dijk et al., 2002). Furthermore, this could enable the prediction of final sugar content in potatoes after cooking and be related to consumer preferences for potato quality.

An important group of phytochemicals in potato tubers are carotenoids, which function as precursors to vitamin A and were decreased upon cooking in all germplasm groups except bred native hybrids. Previous studies showed a general decrease of carotenoids in yellow fleshed potato cultivars after boiling (Burgos et al., 2012; Tierno et al., 2015). This is to be expected as carotenoids are easily degraded under thermal, oxidative and light influence (Schieber et al., 2016). The fact that the accessions with the highest carotenoid levels showed the least degradation, indicates their potential as valuable resources for breeding stable carotenoid levels in potato tubers.

Another vitamin found in potatoes is vitamin C (ascorbic acid) and is as easily degraded by heat as carotenoids (Smirnoff et al., 2000). Wild potatoes had on average about 50-75% less ascorbic acid than the other germplasm groups (Table A.1). Very variable changes of ascorbic acid levels, including increases and decreases, were observed after cooking and the advanced breeding lines were the only group with an overall significant decrease of ~30% after cooking.

The lack of overall change of phenolics in all germplasm groups seemed to be varietal- and metabolite-related. Previous publications report both decreases and increases of total phenolic concentrations and antioxidant activity of cooked relative to raw potatoes (e.g. Burgos et al., 2013a). In the current study, over 60% of the genotypes showed increased phenolics levels with no notable distinctions within the germplasm groups and the changes of individual phenolic compounds ranged from a 90% decrease to a up to 5-fold increase (Table A.3). A recent biofortification study revealed phenolic compounds as inhibitors of iron absorption by the human body (Andre et al., 2015). The present metabolite data presents an ideal resource to identify potatoes suitable for such biofortification efforts.

A more detailed view on the individual genotypes/accessions showed that all advanced breeding lines had very similar changes during the cooking process (Fig. A.2). Within the other three germplasm groups a few genotypes/accessions (NM205, NM206, N315, N311 and W410) had distinct metabolite changes upon cooking. Two native hybrids (NM205 and NM206), with the same parents and similar metabolite composition of raw tubers, were explicitly different from other native hybrids with regards to i) most changes overall and ii) increase of organic acids, amino acids referring umami flavour (e.g. aspartic acid, asparagine) and sugars (fructose, glucose and sucrose). These changes were not entirely similar across genotypes and one of the additional changes in NM205 was an almost 40% increase of β-carotene. The native landraces N315 and N311 showed the most pronounced metabolite changes after cooking within the native group which included increase of amino acids and mixed changes of phenolics. The main difference between the two cultivars was increased sugar levels of N315, contrary to the decreased sugar levels of N311. These details about metabolic variation caused by cooking will give valuable information to breeders during their selection process.

## Correlation of phenotypic and chemotypic measurements

In recent years it has been highlighted that an important favourable attribute for breeding is how chemotypic analysis and its findings become readily accessible. So that farmers can be provided with alternative methods to measure nutrient content in or close to the field. Therefore, the metabolite data was compared to phenotypic properties of the tuber (flesh colour and dry matter content) observed during preparation (Fig. 4). All comparisons between the phenotypic and chemotypic measurements showed a significant correlation. The comparison of metabolites to the tuber flesh colour included only colour conferring metabolites such as carotenoids and phenolics. The regression coefficients emphasised that carotenoids (R=0.45) had a higher positive correlation with tuber colour than phenolics (R=0.39). Furthermore, the analysis highlighted that a secondary red/purple tuber colouration, conferred by anthocyanins, complicates the prediction of phytochemical levels as they can mask the actual colouration derived from carotenoids and “yellow” phenolics such as rutin. Additionally, the biosynthesis of anthocyanins can alter the carbon flow through the phenolics pathway and change the overall phenolic profile as observed in sweet potatoes (Wang et al., 2018). Another feature similar to sweet potato is that the dry matter and total sugar content had a negative correlation leading to the prediction that potatoes with more starch are less sweet, even after boiling (Drapal et al., 2019).

# Conclusion

The metabolite profiling of a potato diversity panel provided a broad overview of the metabolic plasticity of potato tubers. Clear differences in metabolic composition were detected between germplasm groups and gave an indication why certain wild potatoes were preferred during domestication (e.g. decrease of glycoalkaloid and phenolic levels after cooking). Furthermore, the present study highlighted which metabolites with nutritional properties are available in potatoes and how they change after a cooking treatment. The metabolic differences between raw and cooked tubers were in general specific to the germplasm groups and highlighted a few cultivated genotypes with distinct changes. One of the group specific properties was no significant change of carotenoid levels after cooking in the native hybrids bred for high carotenoid content. The positive correlation detected between chemotype (metabolites) and phenotype (tuber colour) is of great advantage for assessment of potato tubers with simple techniques such as spectroscopy. However, for a precise prediction from spectroscopic readings, a more detailed correlation analysis with a larger data set would need to be performed. Nevertheless, for future breeding purposes this metabolite data can be used i) to establish quantifiable metabolite markers for quality potatoes (e.g. lack of browning); and ii) in combination with sensory studies, to quickly screen for potato tubers preferred by consumers (taste and flavour).

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# Declaration of interest

Declarations of interest: none.

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# Tables

**Table 1.** Genotypes analysed in the present study.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Germplasm group** | **Lab** **Code** | **CIP Number** | **Species/subspecies** | **Accession name** | **Tuber colour** |
| **Wild** | W401 | CIP761820 | bukasovii | Juz. Ex Rybin | - |
|  | W402 | CIP762068 | ambosinum | Ochoa | - |
|  | W403 | CIP762183.001 | candolleanum | Berthault | - |
|  | W404 | CIP762183.002 | candolleanum |   | - |
|  | W405 | CIP762418.001 | multiinterruptum |   | - |
|  | W406 | CIP762418.002 | multiinterruptum |   | - |
|  | W407 | CIP762832 | sparsipilum |   | - |
|  | W408 | CIP762866 | tacnaense |   | - |
|  | W409 | CIP763010 | bukasovii | Juz. Ex Rybin | - |
|  | W410 | CIP763693 | incamayoense |   | - |
| **Native** | N301 | CIP700313 | Stenotonum | Cuchipa Ismaynin | cream |
| **landraces** | N302 | CIP701882 | Goniocalyx | Maria Cruz | cream |
|  | N303 | CIP702199 | Stenotonum | Chuco Pitiquiña | cream |
|  | N304 | CIP702467 | Goniocalyx | Milagro | white |
|  | N305 | CIP702547 | Stenotonum | Jancko Fiñu | cream |
|  | N306 | CIP702815 | Stenotonum | Morar Nayra Mari | cream |
|  | N307 | CIP703515 | Phureja | Unknown | yellow |
|  | N308 | CIP703570 | Phureja | Unknown | yellow |
|  | N309 | CIP703654 | Phureja | Chaucha Amarilla | yellow |
|  | N310 | CIP703774 | Stenotonum | Unknown | white |
|  | N311 | CIP703874 | Goniocalyx | Kashapa Tuktun | cream |
|  | N312 | CIP704068 | Goniocalyx | Alqa Coillu | white |
|  | N313 | CIP704243 | Stenotonum | Girancho | cream |
|  | N314 | CIP704771 | Stenotonum | Wila Fiñu | cream |
|  | N315 | CIP705499 | Stenotonum | Cuzqueña | cream |
| **Bred native** | NM201 | CIP311083.005 |  | M3-15.5 | red/yellow |
| **hybrids** | NM202 | CIP311103.030 |  | M3-47.30 | yellow/red |
|  | NM203 | CIP311339.002 |  | M3-18.2 | yellow/purple |
|  | NM204 | CIP311339.114 |  | M3-18.114 | red/yellow |
|  | NM205 | CIP311420.014 |  | M3-10.14 | intense yellow |
|  | NM206 | CIP311420.019 |  | M3-10.19 | intense yellow |
|  | NM207 | CIP311420.074 |  | M3-10.74 | cream |
|  | NM208 | CIP311422.014 |  | M3-26.14 | red/yellow |
|  | NM209 | CIP311422.019 |  | M3-26.19 | intense yellow/purple |
|  | NM210 | CIP311422.033 |  | M3-26.33 | red/yellow |
|  | NM211 | CIP311575.064 |  | M3-19.64 | intense yellow/red |
|  | NM212 | CIP311575.103 |  | M3-19.103 | intense yellow/purple |
|  | NM213 | CIP311623.075 |  | M3-21.75 | intense yellow |
|  | NM214 | CIP311623.105 |  | M3-21.105 | yellow/purple |
|  | NM215 | CIP311623.123 |  | M3-21.123 | intense yellow |
| **Advanced** | V101 | CIP390637.1 |  |   | cream |
| **breeding** | V114 | CIP390478.9 |  | Tacna | cream |
| **lines** | V106 | CIP301023.15 |  |   | cream |
|  | V109 | CIP302498.70 |  |   | cream |
|  | V115 | CIP398193.553 |  |   | cream |
|  | V104 | CIP398208.29 |  |   | cream |
|  | V108 | CIP398208.33 |  |   | cream |
|  | V117 | CIP398098.119 |  |   | cream |
|  | V124 | CIP398098.57 |  |   | cream |
|  | V113 | CIP304399.15 |  |   | cream |
|  | V121 | CIP300072.1 |  |   | cream |
|  | V111 | CIP304350.100 |  |   | cream |
|  | V107 | CIP302499.30 |  |   | cream |
|  | V119 | CIP394223.19 |  |   | cream |
|  | V118 | CIP392797.22 |  | UNICA | cream |
|  | V127 | CIP398098.205 |  |   | pale yellow |
|  | V126 | CIP304349.8 |  |   | pale yellow |
|  | V103 | CIP302476.108 |  |   | pale yellow |
|  | V112 | CIP397077.16 |  | Alliance | pale yellow |
|  | V123 | CIP304405.42 |  |   | pale yellow |
|  | V135 | CIP380389.1 |  | Canchan | pale yellow |
|  | V132 | CIP800048 |  | Desiree | pale yellow |
|  | V136 | CIP391691.96 |  | Serranita | pale yellow |
|  | V129 | CIP720064 |  | Yungay | pale yellow |
|  | V102 | CIP302428.20 |  |   | white |
|  | V110 | CIP304383.80 |  |   | white |
|  | V125 | CIP392780.1 |  |   | white |
|  | V122 | CIP395448.1 |  |   | white |
|  | V116 | CIP379706.27 |  |   | white |
|  | V128 | CIP388676.1 |  | Maria Bonita-INIA | white |
|  | V105 | CIP398203.509 |  |   | yellow |
|  | V120 | CIP394611.112 |  |   | yellow |

**Table 2.** Metabolite levels detected in raw tuber and their change after cooking. Values displayed are the sum of each chemical class averaged by germplasm groups (µg/g DW). Significant changes of metabolite groups after cooking are displayed as percentage increase (↑) and decrease (↓).



# Figure legends

**Fig. 1.** PCA analysis of raw and cooked potato tubers. The analysis was based on the metabolites identified in four replicates as described in the methods section.

**Fig. 2.** Dendrograms based on metabolite composition of raw tubers within each germplasm group. The individual dendrogram show advanced breeding potatoes (A), bred native hybrids (C), native landraces/cultivars (D) and wild potatoes (B). Coloured frames indicate the same taxon: S. candolleanum (red), S. brevicaule (blue), S. multiinterruptum (yellow), S. medians (green); icons indicate same S. tuberosum L. Andigenum group: Stenotonum (box), Goniocalyx (triangle), Phureja (dot); and underlined letters (a-e, y-z) indicate full siblings.

**Fig. 3.** Heat map including dendrogram of raw and cooked (CKD) tubers of all germplasm groups. The germplasm groups included wild potatoes (W), native landraces/cultivars (N), bred native hybrids (NM), and advanced breeding lines (V). Values for each germplasm group were averaged for the display.

**Fig. 4.** Correlation analysis between phenotypic and chemotypic traits. The upper graphs display the correlation between total phenolics, total carotenoid content and the combination of both (total pigment) versus the observed tuber colour in raw tubers. The two lower graphs show the correlation between dry matter content and total sugar content for raw and cooked potatoes separately. The calculated linear correlation coefficient (R2) and significance of the linear regression are displayed for each graph respectively. Data can be found in Table A.4.