Functional ingredients in cooked Colombian diploid potatoes

Liyao Ji

Department of Plant Science

McGill University, Montreal

Macdonald Campus of McGill University

21,111 Lakeshore Road, Sainte-Anne-de-Bellevue, Quebec, Canada

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Abstract

Potato, due to its high consumption worldwide, can be exploited for the delivery of functional ingredients, especially for the poor who do not take any supplements. In this study, the functional ingredients-related metabolites in cooked tubers of eight diploid potato genotypes (*Solanum tuberosum* Group *phureja*) from Colombia were explored. The plants were grown under field conditions in a randomized block design. A total of 24 samples (8 genotypes*3 replications) were used in this study. Tubers were harvested, lyophilized, stored at -80℃ and boiled for 30 minutes. Metabolites were extracted from flesh samples using 60% aqueous methanol with 0.1% formic acid and analyzed using liquid chromatography and high resolution mass spectrometry (LC-HRMS) system. The data files were processed using MZmine-2 software. The peaks were putatively identified based on monoisotopic mass with accurate mass error (AME)<5ppm and fragmentation patterns. Statistical analysis (ANOVA) was done to identify significant metabolites. A total of 294 metabolites were putatively identified and these metabolites belonged to different chemical groups such as phenylpropanoids, flavonoids, alkaloids, fatty acids, and terpenoids. The functional ingredients properties of these identified metabolites were searched in databases and literature. A total of 87 metabolites were associated with health-benefiting roles for humans, such as anticancer, anti-inflammatory, antioxidant, antitumor, antimicrobial, and antiviral. The fold change in abundance of each metabolite was calculated relative to its abundance in the genotype AC04. The natural occurrence of compounds in cooked potato was confirmed based on in-house metabolite library of uncooked potato genotype, Shepody. Several metabolites such as chlorogenate, N-Feruloyltyramine, linoleic acid, and

γ-Linolenic acid were detected in cooked potato as reported from uncooked potato. This study reported several metabolites with functional ingredients values. The metabolites identified here were ranked based on relative fold change in abundance of metabolites in eight genotypes. The metabolites detected in high fold change and with functional ingredients properties can be used as candidates in Colombian potato breeding programs to improve functional ingredients in the indigenous communities' cultivars.

Résumé

Pommes de terre, en raison de sa forte consommation dans le monde, peut être exploitée pour la livraison des aliments fonctionnels, en particulier pour les pauvres qui ne prend pas de suppléments. Dans cette étude, les métabolites liés à les aliments fonctionnels dans les tubercules cuits de huit génotypes de pommes de terre diploïdes (*phureja* de *Solanum tuberosum* Groupe) de Colombie ont été explorées. Les plantes ont été cultivées dans des conditions de terrain dans un design en blocs aléatoires. Un total de 24 échantillons (8 génotypes*3 répétitions) ont été utilisés dans cette étude. Les tubercules ont été récoltés, lyophilisée, stockée à -80 ℃ et bouilli pendant 30 minutes. Les métabolites ont été extraits à partir des échantillons de chair en utilisant 60% de methanol aqueux avec de l'acide formique à 0.1% et analysé par chromatographie liquide et la spectrométrie de masse à haute résolution (LC-HRMS) système. Les fichiers de données ont été traitées en utilisant le logiciel MZmine-2. Les pics ont été identifiés sur la base putative sur la masse monoisotopique avec l'erreur précise de masse (TEA) <5 ppm et motifs de fragmentation. L'analyse statistique (ANOVA) a été fait pour identifier des métabolites importants. Un total de 294 métabolites ont été putativement identifié et ces métabolites appartenait à différents groupes chimiques tels que les flavonoïdes, les phénylpropanoïdes, des alcaloïdes, des acides gras, et terpénoïdes. Les propriétés de ces métabolites identifiés alimentaires fonctionnels ont été recherchés dans les bases de données et de la littérature. Un total de 87 métabolites ont été associé à des rôles bénéfiques pour la santé pour les humains, comme anticancéreux, anti-inflammatoire, antioxydant, anti-tumorale, antimicrobien et antiviral. Le fold change de l'abondance de chaque métabolite a été calculé par rapport à son abondance

dans le AC04 de génotype. La présence naturelle de composés dans la pomme de terre cuite a été confirmé sur la base de la bibliothèque du génotype de pomme de terre crue, Shepody métabolite en interne. Plusieurs métabolites tels que Chlorogenate, N-Feruloyltyramine, l'acide linoléique et l'acide γ-linolénique ont été détectés dans la pomme de terre cuite comme indiqué à partir de la pomme de terre crue. Cette étude fait état de plusieurs métabolites avec des valeurs d'aliments fonctionnels. Les métabolites identifiés ici ont été classés en fonction de rapport fold change de l'abondance des métabolites dans huit génotypes. Les métabolites détectés en haute fold change et avec des propriétés des aliments fonctionnels peuvent être utilisés en tant que candidat dans les programmes de pomme de terre d'élevage colombiennes pour améliorer les aliments fonctionnels dans les cultivars des communautés autochtones.

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Preface and Contribution of Authors to Manuscripts

The presentation of this thesis is in a standard format and is prepared according to McGill University's 'Guidelines for Thesis Preparation'.

I contributed to all the chapters presented in this thesis, my work includes background research, performing all experiments, collecting results, analyzing data and preparing for the thesis. My supervisor Dr. Ajjamada Kushalappa developed the project, provided me with research funds throughout my studies. He contributed to the experimental design, supervision of my study and preparation of this thesis. He edited, corrected all the information presented in this thesis. Dr. Teresa Mosquera provided funds for the research in Colombia. Dr. Ernesto Rodriguez bred the potato, crossed several lines and produced new cultivars. Ms Clara Piñeros-Niño prepared cooked potato samples. Dr. Stan Kubow improved the manuscript. Dr. Danielle Donnelly gave many suggestions for the preparation of this thesis.

List of Abbreviations

AME	Accurate mass error
ANOVA	Analysis of variance
CE-MS	Capillary electrophoresis-mass spectrometry
CML	Chronic myeloid leukemia
DW	Dry weight
FC	Fold change
FW	Fresh weight
GC-MS	Gas chromatography–mass spectrometry
GLA	γ -Linolenic acid
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
LAP	Lapatinib
LC-HRMS	Liquid chromatography and high resolution mass spectrometry
LC-MS	Liquid chromatography-mass spectrometry
LC-NMR-MS	Liquid chromatography-nuclear magnetic resonance-mass spectrometry
LDL	Low-density lipoprotein
m/z	Mass-to-charge ratio
NMR	Nuclear magnetic resonance
NPACT	Naturally Occurring Plant-based Anti-cancer Compound-Activity-Target database

PMN	Plant Metabolic Network
PYVV	Potato yellow vein virus
RCBD	Randomly Complete Block Design
S/N	Signal to noise
UPLC	Ultra-performance Liquid chromatography

Chapter I. Introduction

1.1 General introduction

Potato (*Solanum tuberosum* L.) is an important tuber food crop for human consumption around the world. It originated in South America over 10,000 years ago (CDC 1999a) but did not spread to Europe until late 1500s (CDC 1999b). Now, there are more than 4000 edible varieties of potato which play an important role in contributing to world food security (CIP 2014). Potato is the fourth most important staple crop for humans behind maize, rice and wheat (FAOSTAT 2014) . More than 1 billion people consume potato in the world and the global production has exceed 300 million metric tons (CIP 2014). Most of the potato grown around the world is tetraploid, and the diploid potato (*S. tuberosum* Group *phureja*) is grown mainly in the Andean regions of Colombia, where it is mainly consumed as soup by the indigenous communities.

Potato has the potential to contribute to world food security by providing food to the poor and also by providing food with nutritional value to the developed countries. In comparison with other food crops, potato has higher yield per unit growing area. In North America, potato has a yield of 40.6 mt ha^{-1} on an average which is higher than in any other places (Camire et al. 2009). Besides, potato has a great effect on nutrition and health around the world. Potato contains high content of carbohydrates, protein, dietary fiber, minerals and vitamins and has been shown to contribute to the prevention of nutrient deficiencies. Interestingly, the potato also contains a large number of functional ingredients which can contribute to human health (Ezekiel et al. 2013). Functional ingredient is defined as a

diverse group of compounds that can have beneficial effects on human health (Kruger and Mann 2003). For example, potato is a good source of polyphenols, and contains 530 to 1770 μg/g phenolics (Al‐Saikhan et al. 1995). Phenolics have become interesting functional ingredients mainly because of their antioxidants capacities. Condensed tannins have been shown to have anticarcinogenic, cardiovascular, cholesterol-lowing properties (Gondim Junior et al. 2005, Dykes and Rooney 2006). Avenanthramides are shown to have the capacity of anti-inflammatory and antioxidant (Bratt et al. 2003). Lignans are believed to have risk-reducing properties of breast cancer, and prostate cancer (Cotterchio et al. 2006, Hooper and Cassidy 2006). Alkylresorcinols are believed to have antibacterial and antifungal properties (Ross et al. 2004). Potato is also a good source of flavonoids. Among potato varieties, the flavonoid concentrations vary between 200 and 300μg/g FW (Lewis et al. 1998). Quercetin has been shown to play a role in protecting against cardiovascular disease (Finotti and Di Majo 2003), diabetes, and human immunodeficiency virus (HIV) (Li et al. 2000). Baicalin was recently shown to have the capacity of anti-inflammatory and anti-HIV-1 (Li et al. 2000). Potato is a good source of carotenoids, and the main carotenoids present are lutein, zeaxanthin, violaxanthin and neoxanthin. Carotenoids can play an important role in human health. β-Carotene is valued for the capacity to reduce the risks of lung cancer (Ziegler et al. 1996). Lycopene has been reported to be able to reduce the risks of breast cancer (Zhang et al. 1997) and prostate cancer (Mills et al. 1989, Giovannucci et al. 1995). Lutein and zeaxanthin have been proposed to have the capacity to protect eye diseases (Snodderly 1995, Yeum et al. 1995). Anthocyanin fraction from the potato cultivar CO112F2-2 showed a potent ability of inducing apoptosis in prostate cancer cells

(Reddivari et al. 2007). The anthocyanins from purple potato showed an ability of repressing the growth of stomach cancer cells. Besides, some contents in potato has been shown to play a role in preventing breast cancer (Kallio et al. 2008), colon and liver cancer (Lee et al. 2004), lung cancer (Shih et al. 2007), cardiovascular disease by lowering cholesterol levels (Camire et al. 2009).

Metabolomics has become an excellent tool to detect a large number of biochemical compounds. Fiehn in 2001 defined metabolomics as 'the comprehensive and quantitative analysis of all small molecules in a biological system' (Fiehn, 2001). Metabolomics has recently been applied to different disciplines enabling comprehensive large-scale analyses of various biochemical compounds. Nuclear magnetic resonance (NMR) and mass spectrometry (MS) are currently the two main techniques used in metabolomics studies. NMR spectroscopy is used for low molecular metabolites to get their profiles. Mass spectroscopy has been widely used to analyze large population of metabolites. Liquid chromatography-mass spectrometry can be employed to analyze the thermally labile or involatile molecules (Lu et al. 2008); gas chromatography-mass spectrometry (GC-MS) can be used to profile volatile molecules and derivatized metabolites (Pasikanti et al. 2008). Due to the fast developments in metabolomics technology in general, hundreds of non-targeted metabolites have been identified and measured (Allwood and Goodacre 2010). Liquid chromatography-Mass spectrometry (LC-MS) based approache has an important application in plant metabolites research owing to the detection of diverse plant metabolites. LC-MS approach can be employed mainly to detect the semi-polar plant metabolites (Tolstikov et al. 2003, De Vos et al. 2007). Some groups of plant secondary metabolites such as alkaloids,

phenolic acids, flavonoids, polyamines can be detected by LC-MS (Moco et al. 2006, Rischer et al. 2006). Recently lots of metabolites against pathogen stress have been identified by using a non-targeted metabolomics approach based on liquid chromatography and high-resolution mass spectrometry (LC-HRMS) (Pushpa et al. 2014, Yogendra et al. 2014). However, there lacks studies which investigated the functional ingredients related metabolites in tubers, especially the diploid potato, using LC-HRMS. Therefore, the objective of this study was to identify the functional ingredients related metabolites in cooked diploid potato cultivars from Colombia based on non-targeted metabolic profiling.

1.2 General hypotheses

1.2.1 Many of the secondary metabolites present in potato genotypes have human health benefiting properties.

1.2.2 Potato genotypes varying in agronomic qualities and environmental stress or disease resistance also vary in functional ingredients related metabolites.

1.2.3 The genotypes which contain high fold change of metabolites could be used in the breeding programs to increase the functional ingredients-related metabolites in potatoes through breeding.

1.3 General objectives

1.3.1 To identify metabolites in diploid potato genotypes, derived from a Columbian breeding program intended to improve yield and disease resistance

1.3.2 To find out the functional properties of the identified metabolites published in the literature.

1.3.3 To group the metabolites according to their chemical groups and functional properties

1.3.4 To provide information on functionality of improved diploid potato genotypes and rank cultivars for the production of each metabolite for their use as candidates in Colombian breeding programs.

Chapter II Literature Review

2.1 General information about the importance of potato

Potato is a very important food crop for human consumption around the world. Now there are more than 4000 varieties of potato around the world (CIP 2014). Potato has the potential to contribute to the world food security by providing food to the poor and also by providing food with the nutritional value to the developed countries. In comparison with other food crops, potato has a higher yield per unit growing area. In North America, potato has a yield of 40.6 mt ha^{-1} on average which is higher than any other places (Camire et al. 2009). Besides, potato has a great effect on nutrition and health around the world. Potato contains high content of carbohydrates, protein, dietary fiber, minerals and vitamins and has been shown to contribute to the prevention of nutrient deficiencies. It contains high content of vitamin C and contains about half of the daily requirement for the ascorbic acid of the United States and approximately 20% of the European requirement (Love and Pavek 2008). Moreover, it contains a large amount of B-group vitamins such as folic acids. Some cultivars can play an important role in preventing deficiencies of some minerals such as Zinc and Iron (Burgos et al. 2007). Potato has shown important antioxidant activities such as superoxide scavenging capacity and contains antioxidants like polyphenolic compounds, carotenoids and ascorbic acid (Leo et al. 2008). These metabolites have an important effect on the prevention of cancers. Anthocyanin fraction from the cultivar CO112F2-2 showed a potent ability of inducing apoptosis in prostate cancer cells (Reddivari et al. 2007). The anthocyanins from purple potato showed an ability of repressing the growth of stomach

cancer cells. Besides, potato has been shown to play a role in preventing breast cancer (Kallio et al. 2008), colon and liver cancer (Lee et al. 2004), lung cancer (Shih et al. 2007), cardiovascular disease by lowering cholesterol levels (Camire et al. 2009). However, information on the specific metabolites involved in these is not clearly defined.

2.2 Functional ingredient concept and definition

Functional ingredient is defined as a diverse group of compounds that are intended to produce human health benefits, for example, carotenoids and flavonoids found in fruits and vegetables (Kruger and Mann 2003). Functional ingredients can be single component ingredients, or components of complex mixtures or products derived from novel sources or processes (Kruger and Mann 2003). Unlike the classic direct food additives which are added to food because of their nutritional value, the functional ingredients exert their beneficial properties by the biological or physiological activities within the body (Roberfroid 2000, Kruger and Mann 2003). Thus, the term 'functional' is not meant to differentiate from other food components which are indeed bioactive constituents like nutrients but to convey the functions of ingredients which can play a beneficial role by the physiological activities in the body, as antioxidants, as modulators of biochemical reactions and signal transduction systems, and as regulators of gene expression (Paliyath and Shetty 2011). Functional ingredients can be marketed as food supplements or safe ingredients included in functional foods (Kruger and Mann 2003). Health Canada's definition for functional foods is 'similar in appearance to, or may be, a conventional food that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease

beyond basic nutritional functions, i.e. they contain bioactive compounds' (HealthCanada 1998). In this study, examples of functional ingredients include secondary metabolites such as carotenoids, plant sterols, polyphenols, terpenes, dietary fibre, saponines, glucosinolates and phyto-estrogens and their health benefits include but not limit to anticancerogenous, antimicrobial, antioxidant, immunomodulatory, anti-inflammatory, blood pressure regulation and cholesterol level lowering effects (Wang and Bohn 2012).

2.3 Functional ingredients and their health benefits

Food we eat contain several health benefiting functional ingredients, for example, phenolics, flavonoids, folates, polyamines, anthocyanins, and carotenoids.

2.3.1 Phenolics

Phenolics are the main antioxidants present in our diet (Manach et al. 2004). Phenolics have been shown to have a number of health benefits. Potato is a good source of polyphenols, and contains 530 to 1770 μg/g phenolics (Al‐Saikhan et al. 1995). Potato has been reported to contain lignin, coumarins, anthocyanins and flavones, tannins, monohydric phenols and polyhydric phenols (Talburt et al. 1987). Chlorogenic acid was reported as the predominant phenolic compound in potato (Mattila and Hellström 2007). Potato is also a good source of flavones aglycones which are potent antioxidants (Ezekiel et al. 2013).

Phenolics have become interesting functional ingredients mainly because of their antioxidants capacities. Epidemiological studies have reported that phenolics are able to reduce the risks of cardiovascular diseases (Hertog et al. 1995, Hertog et al. 1997) and several kinds of cancers (Hertog et al. 1994, Hertog et al. 1995). Condensed tannins have

been shown to have anticarcinogenic, cardiovascular, cholesterol-lowing properties (Gondim Junior et al. 2005, Dykes and Rooney 2006). Avenanthramides are shown to have the capacity of anti-inflammatory and antioxidant (Bratt et al. 2003). Lignans are believed to have risk-reducing properties of breast cancer, and prostate cancer (Cotterchio et al. 2006, Hooper and Cassidy 2006). Alkylresorcinols are believed to have antibacterial and antifungal properties (Ross et al. 2004).

2.3.2 Flavonoids

Over 5,000 flavonoid metabolites have been identified (Yao et al. 2004). In potato varieties, flavonoid metabolite concentrations vary between 200 and 300μg/g FW (Lewis et al. 1998). Some compounds such as catechin, epicatechin, erodictyol, kaempeferol and naringenin belong to the chemical group of flavonoids (Brown 2005). Potato also contains large amount of anthocyanins whose content ranged from 5.5 to 35mg/100g FW (Brown 2008).

Flavonoids have been shown to have many health benefits, such as, antioxidant, anticancer, anti-allergic, anti-inflammatory (Ren et al. 2003). Quercetin has been shown to play a role in protecting against cardiovascular disease (Finotti and Di Majo 2003), diabetes, and human immunodeficiency virus (HIV) (Li et al. 2000). Baicalin was recently shown to have the capacity of anti-inflammatory and anti-HIV-1 (Li et al. 2000). Flavonoids were reported to be able to reduce the risk of coronary artery disease (Naderi et al. 2003). In addition, flavonoids have been shown to be able to prevent oxidation of LDL (Duthie et al. 2000).

2.3.3 Folate

Folate is a water-soluble vitamin which represents an important nutritional component in the human diet. Folate contents in potato ranged from 12 to 37μg/100 g F.W. (Konings et al. 2001). The folate contents in potato are not significantly high, but similar to flavonoids, it should be considered to be a source of great value due to the high consumption of potatoes. Folate has been shown to play a role in colorectal cancer prevention (Hubner and Houlston 2008). Folate was reported to be able to reduce the risk of some cancers and cardiovascular diseases (Bailey et al. 2003).

2.3.4 Polyamines

Polyamines (putrescine, spermidine, and spermine) are of great importance to human health. They are essential components of living cells and have great effect on cellular metabolism (Okamoto et al. 1997). A concentration of 200 nmol/g of putrescine and 93 nmol/g of spermidine have been reported in potato (Okamoto et al. 1997). Polyamines have an effect on cellular proliferation, apoptosis and gut maturation (Larqué et al. 2007). Polyamines may have an effect on immune system. It is reported that polyamines have a suppressor effect on the pulmonary immunologic responses (Moinard et al. 2005).

2.3.5 Carotenoids

Carotenoids are lipid-soluble compounds which have been considered to have many health benefits. Carotenoids have been reported to play a role in eye protection, cardiovascular disease and cancers. Potato is a good source of carotenoids and the main carotenoids present

are lutein, zeaxanthin, violaxanthin and neoxanthin. The yellow-fleshed potato cultivars showed more carotenoid contents than white-fleshed potatoes. Carotenoids concentration ranged from 50 to 350μg/100 g FW in white-fleshed cultivars and from 800 to 2000μg/100 g FW in yellow-fleshed cultivars (Brown 2008).

Carotenoids have been proposed for playing a role in human health. β-Carotene has shown the capacity to reduce the risks of lung cancer (Ziegler et al. 1996). Lycopene has been reported to be able to reduce the risks of breast cancer (Zhang et al. 1997) and prostate cancer (Mills et al. 1989, Giovannucci et al. 1995). Lutein and zeaxanthin have been proposed to have the capacity to protect eye diseases (Snodderly 1995, Yeum et al. 1995).

2.4 Metabolomics and its application in functional ingredients

Metabolomics is 'the nonbiased identification and quantification of all metabolites in a biological system' (Ellis et al. 2007). Nuclear magnetic resonance (NMR) and mass spectrometry are the two main technology platforms used in metabolic profiling.

2.4.1 NMR Spectroscopy

NMR spectroscopy is generally used to profile low molecular weight metabolites. NMR spectroscopy result is highly reproducible and quantitative. Due to the high existence of hydrogen in low molecular weight metabolites, ^{1}H NMR spectroscopy is most frequently employed. Besides, NMR spectroscopy can detect ^{13}C and ^{15}N as well as ^{31}P. NMR spectroscopy has many advantages, for example, it doesn't need sample preparation and it can be used to analyze any kind of biofluids. For NMR it is possible to use the entire tissue for metabolic profiling (Tsang et al. 2005). However not only the diversity of metabolites

detected in NMR is limited but also it is not suitable to detect several plant metabolites that are in low concentrations.

2.4.2 Mass Spectrometry

Mass spectrometry has been widely used to analyze large population of metabolites benefited by its high sensitivity, selectivity and wide dynamic range. Although it can directly analyze the low molecular weight metabolites, it has problems in detecting high molecular weight compounds (Villas- Bôas et al. 2005, Dettmer et al. 2007). Liquid chromatography-mass spectrometry can be employed to analyze the thermally labile or non-volatile molecules (Lu et al. 2008); gas chromatography-mass spectrometry (GC-MS) can be used to profile volatile molecules and derivatized metabolites (Pasikanti et al. 2008). First, using this technique may cause some losses of certain compounds because it requires metabolites to be extracted using organic solvents. Besides, it requires some metabolites to undergo chemical derivatization procedures before they can be analyzed.

2.4.3 Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid chromatography-Mass spectrometry (LC-MS) based approach has an important application in plant metabolites research owing to the great diversity of plant metabolites. LC-MS approach can be employed to detect the semi-polar compounds of plant metabolites (Tolstikov et al. 2003, De Vos et al. 2007).

Some groups of plant secondary metabolites such as alkaloids, phenolic acids, flavonoids, polyamines can be detected by LC-MS. More advanced approaches such as combining LC with ultra-high-resolution mass spectrometry (Breitling et al. 2006, Peterman et al. 2006)

and tools like LC-NMR-MS (Breitling et al. 2006, Peterman et al. 2006), as well as improved separation technologies such as ultra-performance LC (UPLC) coupled to MS (Laaksonen et al. 2006, Nordström et al. 2006) will benefit the process of identifying diverse plant metabolites. The use of LC-HRMS, such as Orbitrap, has the potential to detect thousands of metabolites (Pushpa et al. 2014, Yogendra et al. 2014).

2.4.4 Application of metabolomics in food improvement

Metabolomics has been demonstrated to be a powerful tool for the analysis and comparison of metabolites for the improvement of food crops. It has a very wide application in food science and nutrition science. The health benefits of most of the foods reported are mainly due to metabolites contained in the food. Metabolites can be used to test the outcome of breeding or genetic engineering on nutritional value of foods (DellaPenna 1999, Trethewey 2004). High-performance liquid chromatography (HPLC) has been used to measure soybean lutein, which showed high variation among 20 different genotypes (Seguin et al. 2011). In raspberry 300 individual plants have been analyzed based on LC-MS approach to identify significant environmental and genetic effects on metabolite concentrations (Stewart et al. 2007). A metabolic analysis using a GC-MS approach on potato was done to detect the variation between cultivars and landraces for breeding purposes (Dobson et al. 2008). The metabolome of two genetic modified tomato lines were compared with the metabolomes of commercial tomato cultivars using GC-MS, LC-MS and CE-MS to identify the significant difference among them (Kusano et al. 2011).

Chapter III Study: Identification of functional ingredients in cooked potatoes

3.1 Rationale

Phytochemicals have been identified in tubers of several potato genotypes (Chong et al. 2013, Lachman et al. 2013). However, reports on their functionality are limited (Thompson et al. 2009, Ezekiel et al. 2013). Metabolomics has become a popular tool to detect hundreds of biochemical compounds in a sample (Kushalappa and Gunnaiah 2013). Thus it is possible to detect novel metabolites which may also have functional ingredients properties.

3.2 Materials and methods

3.2.1 Potato genotypes

The diploid potato genotypes (*Solanum tuberosum* L. Group *phureja*) used in this study were hybrids produced through cross among four Criolla potato cultivars: Criolla Colombia, Criolla Guaneña, Criolla Galeras, and Criolla Latina by the Universidad Nacional de Colombia. The hybrids were selected based on agronomic characteristics, tuber yield potential, and resistance to *Phytophthora infestans*, *Ralstonia solanacearum*, *Spongospora subterranea*, *Rhizoctonia solani* and potato yellow vein virus (PYVV). These hybrid genotypes are of medium height, erect, a vegetative period between 110 and 150 days, spherical tubers with yellow skin and flesh, and shallow-depth eyes. From this collection eight genotypes were selected for this study.

3.2.2 Potato production and sample collection

Eight potato genotypes with good agronomic qualities were planted in field, Nariño, Colombia. Each genotype was grown in three replicates. Recommended fertilization practices were followed. Potato tubers were harvested and stored at 4℃ until use. The tubers were boiled for 30 min, skin pealed, ground in liquid nitrogen, lyophilized and shipped in dry ice to McGill University for further analysis.

3.2.3 Metabolite extraction and analysis

Metabolites were extracted from the lyophilized potato flesh samples using 60% aqueous methanol with 0.1% formic acid (De Vos et al. 2007). Metabolites were analyzed using liquid chromatography and high resolution mass spectrometry (LC-HRMS) system (LC-ESL-LTQ Orbitrap, Thermo Fisher, Waltham, MA, USA) in a negative ionization mode, fitted with a relatively polar reverse phase Kinetex column XB-C18 (5 cm x 2.1 mm) (Phenomenex, AC, USA) (Bollina et al. 2010). Mass resolution was set at 60,000 at 400m/z. First, all samples were run to obtain the MS1 files and then the samples of AC50 and AC51 were re-run to get the MS/MS fragmentations using a normalized collision induced dissociation energy of 35 eV. All the data obtained were recorded in centroid mode (Yogendra et al. 2014).

3.2.4 LC-HRMS output data processing

The data files were converted into mzXML and analyzed using MZmine-2 software for mass detection, chromatogram deconvolution, identification of peaks, and retention time

alignment across the samples (Pluskal et al. 2010). For peak identification, wavelets were used to signal to noise (S/N) threshold of 5, wavelet scales of 0.2 to 5 min, and a peak duration of 0.0 to 5.0. RANSAC alignment was used for retention time alignment with an *m/z* tolerance of 0.001 to 5.0 ppm, a retention time tolerance of 0.5 min, iterations of 10,000, and a threshold value of 0.5 s. The accurate mass, retention time and their relative intensity was imported to MS Excel; peaks that are not consistent among replications and those annotated as isotopes and adducts were eliminated from further analyses.

3.2.5 Identification of metabolites

Putative identification of each metabolite was made based on: i) the monoisotopic mass (MS-1) match with metabolites reported in different databases with an accurate mass error of (AME<5ppm): Plant Metabolic Network (PMN 2013), LIPIDMAPS, and KEGG (Kushalappa and Gunnaiah 2013); ii) MS/MS fragmentation pattern of metabolites in house spiked MS/MS library MASSBANK, METLIN, and MS2T; iii) *in-silico* fragmentation as described previously (Gunnaiah et al. 2012). The metabolites with a putative name of identity were classified according to chemical groups based on referenced databases such as PubChem and PMN (Kushalappa and Gunnaiah 2013).

3.2.6 Functional ingredients related properties of metabolites

Functional ingredients properties of the identified metabolites were searched in databases and literatures. Anticancer related metabolites were confirmed through NPACT database (Mangal et al. 2013).

3.2.7 Statistical analysis

Treatments were replicated three times and assigned to a Randomly Complete Block Design (RCBD). The abundances of metabolites were normalized relative to abundance in the genotype AC04 to derive relative fold change for a given metabolite. All the data were subjected to analysis of variance to compare eight different genotypes and to indicate significance based one way ANOVA using MS Excel. Tukey's test was applied to indicate the statistical significance of each two of the genotypes.

3.3 Results

3.3.1 Metabolic profiles of cooked potatoes

A total of 294 metabolites were detected in cooked potatoes from 8 genotypes. The metabolites belonged to different chemical groups including 20% fatty acids and acyls, 10% terpenoids, 10% flavoniods, 6% alkaloids, 4% amino acids, 4% phenylproponoids (Fig.3.1). The chemical groups of heterocyclic compounds, glycerophospholipids, polyketides, nucleic acids, hydrocarbons, prenol lipids, carbohydrates comprised 9, 8, 5, 4, 4, 3 and 3%, respectively. Other metabolites belonging to chemical groups including sulfur compounds, carboxylic acids, sterol lipids and amides were also detected.

Among the eight genotypes, the highest number of metabolites was identified in genotype AC51 and AC52, with 281 and 280 metabolites, respectively. A total of 278 metabolites were identified in both AC50 and AC63. AC59 and AC64 had 276 and 272 metabolites, respectively. The lowest number of metabolites was identified in AC04 and AC09 with 270 and 271 metabolites, respectively (Fig 3.2).

3.3.2 Functional ingredients related metabolites in cooked Colombian potatoes

Out of 294 metabolites identified in cooked Colombian diploid potatoes from 8 genotypes, 87 were reported to exhibit important human health benefits, including anticancer, anti-inflammatory, antimicrobial, antioxidant, and anti-HIV (Cavin et al. 1998, Chen et al. 2004, Zhu et al. 2012). In the chemical group of phenylpropanoids, out of eight metabolites identified, 3 metabolites have shown health benefits like antidiebetic, antioxidant and anticancer effects. Out of 21 metabolites that were classified as flavonoids, six of them have anticancer effectes, and two have anti-inflammatory and anti-microbial properties. Out of 26 terpenoid metabolites 14 have human health benefits including anticancer, antioxidant, and anti-apoptotic effects. The 49 fatty acids and acyls have exhibited diverse health related benefits such as anti-HIV-1, antimicrobial, antiviral, and anti-inflammatory (Fig 3.3).

3.3.3 Relative abundances of functional ingredients related metabolites among genotypes

Out of 87 metabolites, which have been reported to show human health benefits, only 46 have been previously reported in plants. Both the intensities and fold change of metabolites significantly varied among eight genotypes. The fold change was calculated relative to abundance in the genotype AC04. The genotype AC51 had high fold change of 46 and 80 for the metabolites linolenic acid and γ-Linolenic acid, respectively. The genotype AC63 had the highest fold change of 7 for the metabolite N-Feruloyltyramine. A high fold change of 5 was observed in AC64 for the metabolite Cucurbitacin F (Table 3.1).

3.3.4 Impact of cooking on metabolite structure

The occurrence of compounds detected in cooked potato was confirmed based on in-house metabolite library of compounds detected in uncooked potato, from the genotype Shepody (*Solanum tuberosum* L.). Several metabolites such as chlorogenate, N-Feruloyltyramine, linoleic acid, γ-Linolenic acid, etc. were detected in cooked potato as reported from uncooked potato (Table 3.1).

3.4 Discussion

Potato has the potential to contribute to the world food security by providing food to the poor and also by providing food with increased nutritional value to the developed countries. The indigenous communities in Nariño area of Colombia are impoverished and their food security is precarious. The people there consume potatoes mainly as cooked potato soup rather than French fries or potato chips. In this study, we have identified some of the metabolites which have been reported for their important health benefits and their role in improving the diet and health have been discussed.

3.4.1 Chlorogenate

In our study, it was detected in all of the 8 genotypes, and the abundance of this compound is significantly different among 8 genotypes ($P<0.05$). The concentration of chlorogenate was reported previously to range from 9.65±0.49 to 18.7±12.10 mg/100 g of fresh weight among potato cultivars (Dao and Friedman 1992). In boiled potatoes, the content is 0.319±0.01 mg/g of freeze-dried weight (Dao and Friedman 1992). Chlorogenic acid can

also be found in bamboo, *Phyllostachys edulis* (Kweon et al. 2001) and in many other plants such as apple (mean values 30-60 mgkg-1), berry fruits and grapes (Clifford 1999). It is a very important phenolic compound identified in peach (Cheng and Crisosto 1995) and in prunes (184mg/100g) (Stacewicz-Sapuntzakis et al. 2001). It has also been found in green coffee bean extract (Khalesi et al. 2014). Chlorogenate has been suggested as a potential functional ingredients. It has been reported to show antioxidant properties, for example, it can protect the blood granulocytes from oxidative stress (Bouayed et al. 2007, Marinova et al. 2009), it exhibits scavenging abilities of organic free radicals and peroxy radicals (Kono et al. 1997), it can also increase the resistance of LDL (low density lipoprotein) and exhibit anti-lipidemic and anti-diabetic effects (Shafi and Tabassum 2013). Besides, it shows anti-carcinogenic effects, such as the inhibition of the chemical-induced carcinogenesis in rats and hamsters (Rakshit et al. 2010), anti-proliferative effects on HepG2 cells (Zhang et al. 2012), and apoptosis induction of Bcr-Abl^{+} chronic myeloid leukemia (CML) cell lines (Rakshit et al. 2010).

3.4.2 N-Feruloyltyramine

In the present study, it was detected in all of eight genotypes and the abundance was the highest in AC63 (FC=7.1), and the next highest was AC59 (FC=3.3). The result is of statistical significance ($P<0.05$). It was reported in potato to be associated with the resistance to late blight disease (Yogendra et al. 2014). This compound was previously reported in *Tinospora crispa* Miers to exhibit antioxidant and radical scavenging properties (Cavin et al. 1998). It has also been reported to show strong anti-inflammatory effects

mediated by inhibition of arachidonate 5-lipoxygenase (Al-Taweel et al. 2012). It also exhibits strong anti-nitric oxide (Anti-NO) activity (Yokozawa et al. 2000). It also contributes to the anti-obesity properties because of the inhibition of pancreatic lipase (Ahn et al. 2013).

3.4.3 Tricin

Tricin was detected in AC04, AC09 and AC50 with FC=3.3 compared with AC64. No statistical significance was found among genotypes. Tricin has been reported in rice bran at a concentration of 193.05±0.03 mg/100g (Hudson et al. 2000). It is widely present in Gramineae plants. It was valued for its role in the survival of breast and colon cancer cells (Hudson et al. 2000). It is believed to play the role as a cancer chemopreventive agent in clinical development (Verschoyle et al. 2006). Tricin has been reported to have antioxidant (Hasegawa et al. 2008) and antiviral activities (Sakai et al. 2008). Tricin has also been reported to have potent anti-inflammatory effects and neuroprotective effects (Jung et al. 2014). Tricin from alfalfa showed cytotoxic effects in the mouse tumor cell line (Gatouillat et al. 2014). This compound extracted from rice bran can play a role in inhibiting the growth of human-derived malignant MDA-MB-468 breast tumor cells (Cai et al. 2004) and can interfer with intestinal carcinogenesis in ApcMin mice (Cai et al. 2005). It also exhibited inhibitory effects on the P-glycoprotein in human breast cancer cells (Jeong et al. 2007). Its anticancer effects have been recorded (NPACT 2012).

3.4.4 Aphidicolin

This is the first time to report the presence of aphidicolin in potato. In our study, no statistical significance was found among the abundances of 8 genotypes. Aphidicolin, which was derived from cultures of *Cephalosporium aphidicola* and was proved to be a DNA synthesis inhibitor and by inhibition of DNA polymerase α and δ it exhibited cytotoxic effect in vitro against tumor cells (O'Dwyer et al. 1994).This compound exhibited antitumor activities in transplantable murine tumors in vivo (O'Dwyer et al. 1994). It was reported to exhibit strong cytotoxicity and apoptosis against human leukaemic (HL-60) cells (Gallo et al. 2014). Previous research also showed that when combined with retinoic acid, it can inhibit the proliferation of HL-60 cells more effectively without causing severe cytotoxicity (Chou and Chervenick 1985). It has also been reported to play a role in inhibiting the growth of virus both in tissue culture and in rabbit eyes (Bucknall et al. 1973).

3.4.5 Isobrucein B

In our present study, higher fold changes in abundance was found in AC09 (FC=3.8), AC52 (FC=3.8) and AC59 (FC=3.3). The abundances in these three genotypes are significantly higher than the others ($P<0.05$). Isobrucein B is a compound which is mainly found in the leaves, stems and roots of Passiflora spruce (Silva et al. 2009). It has also been found in Picrolemma sprucei (Simaroubaceae) (Silva et al. 2009), Picrolemma sprucei (Vieira et al. 2014), stems of Brucea mollis (Chen et al. 2013). However, this has not been reported from potato tubers. It is reported to have antiplasmodial (Silva et al. 2009) and anthelmintic effects (Nunomura et al. 2006). It also has gastro-protective activities as proved in mouse

model of NSAID (indomethacin)-induced gastritis (Vieira et al. 2014). Besides, it is proved to exhibit cytotoxic effects against melanoma lines and other tumor lines (Tischler et al. 1992).

3.4.6 Cucurbitacin F

The abundance of this compound varied among genotypes and had the highest in AC64 (FC=5.5). It is significantly higher than the other genotypes ($P<0.05$). This is the first report of cucurbitacin F in tubers of potato. Apart from potato, it has been discovered in other plants such as owania Mexicana (Konoshima et al. 1994a), in seeds of Kageneckia angustifolia (Rosaceae) (Muñoz et al. 2002). Cucurbitacin F exhibited cytotoxic activities against human tumor cells (Chen et al. 2005). It showed modest anticancer activities (De Vos et al. 2007, Lee et al. 2010, NPACT 2012). Cucurbitacin F derivatices can inhibit the HIV-1 replications in H9 cells (Konoshima et al. 1994b). Cucurbitacin F has been shown to significantly inhibit the Epstein–Barr virus activation. (Konoshima et al. 1994b).

3.4.7 Enhydrin

The highest fold change for enhydrin was observed in AC64 (FC=6.5). It is of statistical significance compared with other genoypes ($P<0.05$). This is the first report of enhydrin in cooked potato tubers. It was mainly reported to be found in *Smallanthus sonchifolius* leaves. Enhydrin from *Smallanthus sonchifolius* leaves exhibited in vivo hypoglycemic activity and this can also reduce post-prandial glucose and play a role in the treatment of diabetic animals (Genta et al. 2010). It has been proved to exhibit potent cytotoxicity, by inhibiting cell proliferation and inducing apoptosis in cervical cancer cells (Siriwan et al. 2011). One study

investigated the anti-inflammatory and anti-hyperalgesic effects of enhydrin in the rat carrageenan inflammation model, and concluded that enhydrin could be used in the treatment of inflammatory pain by showing that the hyperalgesic response was significantly blocked and the edema response was significantly attenuated (Feltenstein et al. 2004). In addition, enhydrin showed very good antibacterial activities (Choi et al. 2010).

3.4.8 Linoleic acid

The abundances varied significantly among genotypes tested, with the highest in AC51 (FC=46.3), while the abundances were also high in AC52 (FC=11.0). The abundance in AC51 is significantly higher than the others ($P<0.05$). It can be used in breeding programs to obtain high levels of linoleic acid. This has been reported in leaves of potato (Pushpa et al. 2014, Yogendra et al. 2014). Linoleic acid has been proposed for its effects in protection of mammary and prostate cancer (Belury 2002 a). It is proposed as a functional ingredients mainly because of its antidiabetogenic and anticarcinogenic effects (Belury 2002 b). It also exhibits positive effects on reducing obesity, the formation of bones and immunity system (Belury 2002 b).

3.4.9 γ-Linolenic acid (GLA)

The highest abundance of this compound was found in AC51 (FC=80.5), and was also quite high in AC52 (FC=18.5). It is significantly higher than the others ($P<0.05$). This is the first report of GLA in cooked potato. However, the seed oil of borage (Borago officinalis L.) is the richest plant source of GLA, other available sources of GLA are evening primrose (Oenothera biennis L.) and blackcurrant (Ribes nigrum L.) (Syed Rahmatullah et al. 1994).

The percentage of GLA is up to 17-25% % in seeds of borage, 8-10% in seeds of evening primrose, and 12-20% in blackcurrant (Spurvey and Shahidi 2000).

GLA is one of the essential fatty acids which cannot be manufactured within the body, so it can only be got from food (Horrobin 1992). In healthy people, the need for GLA is no more than 25-100 mg/day; however, it was suggested for the old people to take 125-500 mg per day as a nutritional supplementation (Horrobin 1992). GLA has various human health benefits including anti-inflammation, anti-diebetic, reducing cardiovascular disorders and reproductive disorders (Borgeat et al. 1976). The animal studies showed that GLA can play a role in the inhibition of cancer growth at a dosage of 4-8 g/day or more (Horrobin 1992).

3.4.10 Nutrients and functional ingredients in high abundance in potato

Potato is a very efficient staple food crop according to its abundance of nutrients and functional ingredients. First, compared to cereals, potato contains more dry matter as well as protein per unit growing area (Ezekiel et al. 2013). Cooked potatoes have more protein and calcium compared to maize (CIP 2014). Potato contains a small portion of protein since protein only accounts for 1-1.5% of fresh weight (Ortiz-Medina 2007), however, the protein in potato is of greater value in comparison with other crop proteins (Buckenhüskes et al. 2005). In addition, potatoes contain high contents of non-lignified dietary fiber which can contribute to human health (Lazarov and Werman 1996). An average serving of potato contains fiber around 10% of the daily recommended amount (CIP 2014). Besides, potatoes have a good source of minerals including potassium, phosphorus, and calcium (Burton 1989, Buckenhüskes et al. 2005). Potato contains vitamins including vitamin C and vitamin B

(Brown 2008). One cooked medium size potato provides about 50% of daily requirement of vitamin C for one adult (CIP 2014). Besides, potato is also a good source of phytochemicals. Potato contains phenolic compounds ranging from 530 to 1770 µg/g (Al‐Saikhan et al. 1995). The concentrations of phenols in potato were the highest after apples and oranges (Chun et al. 2005). In addition, potatoes are a good source of carotenoids, white-fleshed potato cultivars contains carotenoids at a content of 50-350 µg/100 g FW, in contrast, its concentration in yellow-fleshed varies between 800 and 2000 µg/100 g FW. Some major carotenoids in potatoes includes lutein, zeaxanthin, violaxanthin and neoxanthin (Brown 2008).

3.4.11 Application of plant metabolic engineering and molecular breeding in increasing the functional ingredients related metabolites

Potato is regarded as a potential source for several functional ingredients because of various secondary metabolites with biological roles. However, not all the secondary metabolites in potato can fulfill the human need. Thus, plant molecular breeding can be applied to increase the content of the desired metabolites in potato. The biotechnologies of molecular biology offer a strategy for enhancing the functional ingredients in plants. Until today, there have been many studies on increasing the nutritional values by increasing the content of nutrients, phytochemicals and metabolites in plants. For example, with an aim of increasing the carotenoids in potato, an overexpression of three genes CrtB, CrtI and CrtY from bacteria resulted in a 20-fold of carotenoids and a 3600-fold of β-carotene in transgenic potato tubers (Diretto et al. 2007). One 250g (FW) transgenic potato can provide half of the daily recommended amount of vitamin A (Diretto et al. 2007). Another study on potato has

successfully generated a transgenic potato by the overexpression of a bacterial phytoene synthase. Its carotenoids content increased from 5.6 to 35 µg/g DW(Ducreux et al. 2005). The transgenic tobacco (*Nicotiana nudicaulis*) plants were generated by constitutive overexpression of the gene CrNCED1 encoding the 9-cis-epoxycarotenoid dioxygenase (NCED) of abscisic acid synthesis. The transgenic plants contained a higher concentration of abscisic acid relative to the wild type (Xian et al. 2014). The overexpression of the UGT73B6 gene encoding 480 deduced amino acid residues led to an increased content of salidroside in *Rhodiola sachalinensis* plant (Ma et al. 2007). Accordingly, in the fatty acid pathway, the overexpression of the genes encoding D6, D12 and D15 fatty acid desaturase resulted in a higher accumulation of ω-3 fatty acids in canola (*Brassica napus*) seeds (Ursin 2003). Similarly, a higher content of β-carotene in tomato fruits was achieved by transferring the CrtI gene from bacteria to tomato. The transgenic plants contained three times increase of β-carotene than wide types (Rosati et al. 2000).

Table 3. 1: Fold change in abundances of functional ingredients metabolites in diploid cooked potato (*Solanum tuberosum* Group *phureja*)

Observed mass[b] (Da)	Exact mass (Da)	Metabolite/chemical group	Health benefits	Genotype[a]/Fold change[c]							
				AC04	AC09	AC50	AC51	AC52	AC59	AC63	AC64
Phenylproponoids											
354.0962	354.0951	Chlorogenate[d]	Antioxiant (Bouayed et al. 2007), Anti-diabetic and anti-lipidemic effects(Shafi and Tabassum 2013)	1.0	1.2	0.9	0.9	0.8	0.7	0.9	0.5
313.1323	313.1314	N-Feruloyltyramine[d]	Antioxidant(Cavin et al. 1998)	1.0	2.5	1.2	1.1	1.0	3.3	7.1	1.2
Flavonoids											
288.0624	288.0634	Dihydrokaempferol	Antimicrobial (Malterud et al. 1985)	1.0	0.9	0.9	0.7	0.9	0.7	0.8	0.6
330.073	330.074	Tricin	Anticancer (NPACT), most potent anti-inflammatory effects(Jung et al. 2014)	1.0	1.0	1.0	0.5	0.7	0.5	0.4	0.3
Terpenoids											
338.244	338.2457	Aphidicolin	Antitumor activity (O'Dwyer et al. 1994), antiviral activity (Bucknall et al. 1973)	1.0	0.8	0.8	0.9	0.8	0.8	0.8	0.8
692.3396	692.3408	Scillaren A	Cardiotonic Agents (Ayobe and Tarazi 1983)	1.0	1.1	1.8	0.9	1.0	1.2	1.0	0.4

522.2115	522.2101	Isobrucein B	antineoplastic agent (Leskinen et al. 1984)	1.0	3.8	1.9	1.5	3.8	3.3	2.8	0.7
414.1173	414.1162	Asperuloside[d]	Anti-inflammatory and immunomodulatory effects (Zhu et al. 2012)	1.0	0.8	0.6	0.4	0.3	1.0	1.2	0.4
518.3259	518.3244	Cucurbitacin F	Anticancer (Chen et al. 2004, Lee et al. 2010, NPACT 2012)	0.0	1.0	1.6	1.1	0.7	2.6	0.8	5.5
356.1118	356.1107	Gentiopicrin	Anti-apoptotic (Doughari 2012)	1.0	1.6	0.0	2.0	0.1	0.8	0.5	0.5
464.1698	464.1682	Enhydrin	Antibacterial activity (Choi et al. 2010)	0.0	1.0	2.3	0.0	1.0	2.4	0.0	6.5
206.1679	206.1671	Alpha-Irone	Antioxidant (Kerschner et al. 2005)	1.0	1.0	1.0	1.5	1.4	1.2	1.8	2.6
Fatty acids											
282.2566	282.2559	(9Z)-Octadecenoic acid[d]	anti-oxidative and diabetic nephropathy (Sales-Campos et al. 2013)	1.0	0.9	0.9	1.2	0.9	1.0	1.1	1.3
270.2566	270.2559	Methyl palmitate[d]	Anti-inflammatory and Antifibrotic (El-Demerdash 2011)	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.1
280.241	280.2402	Linoleic acid[d]	Mammary and prostate cancer protection, (Belury 2002 a), antidiabetogenic, anticarcinogenic (Belury 2002 b)	1.0	1.3	3.7	46.3	11.0	2.3	2.6	3.1

278.225405	278.2246	γ-Linolenic acid[d]	anti-inflammation, anti-diebetic, reducing cardiovascular disorders and reproductive disorders(Borgeat et al. 1976)	1.0	1.6	6.9	80.5	18.5	3.3	2.4	3.4
200.1784	200.1776	Dodecanoic acid[d]	Antibacterial activity (Ouattara et al. 1997)	1.0	0.9	0.9	1.0	1.0	1.0	1.1	1.2
158.1315	158.1307	Nonanoic acid[d]	Antifungal Agents (Aneja et al. 2005)	1.0	0.9	0.9	0.9	0.9	0.9	0.9	1.0
256.2407	256.2402	Hexadecanoic acid[d]	Inhibition of HIV-1 infection, (Reshef et al. 1997), type 2 antidiabetic(Mathur et al. 2011)	1.0	0.9	0.9	1.3	1.0	1.0	0.7	1.3
188.1057	188.1049	Azelaic acid	Antineoplastic Agents(Ferioli et al. 1994)，Dermatologic Agents(Graupe et al. 1996)	1.0	1.5	0.9	3.1	0.9	1.5	1.8	1.5
186.1628	186.162	Undecanoic acid	Antitumoral activity (Townsend et al. 1961)	1.0	1.0	1.0	1.0	1.0	1.1	1.0	1.1

[a]Potato genotypes (*Solanum tuberosum* Group *phureja*) used: AC04, AC09, AC50, AC51, AC52, AC59, AC63, and AC64.

[b]Mass (m/z) observed corrected for H (1.0078) as it was analyzed in negative mode.

[c]Normalized values or fold change relative to abundance in genotype AC04. If the metabolite is absent in AC04, then the fold change was calculated relative to abundance in genotype AC09.

[d]The presence of the metabolites was checked in the in-house library of uncooked potato on Shepody

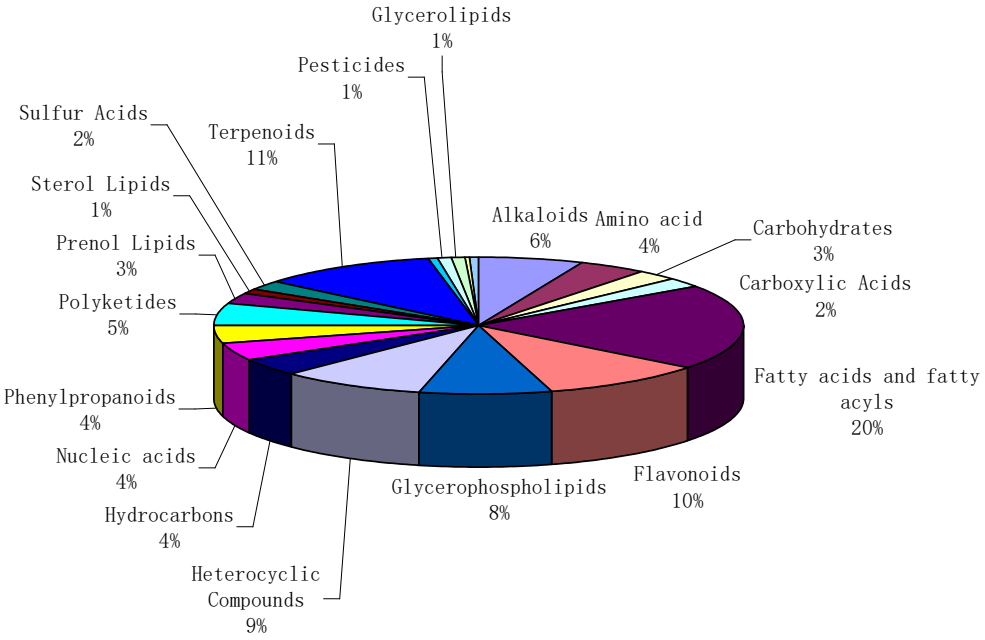

Figure 3. 1: Composition of chemical groups of metabolites in 8 cooked diploid potato genotypes.

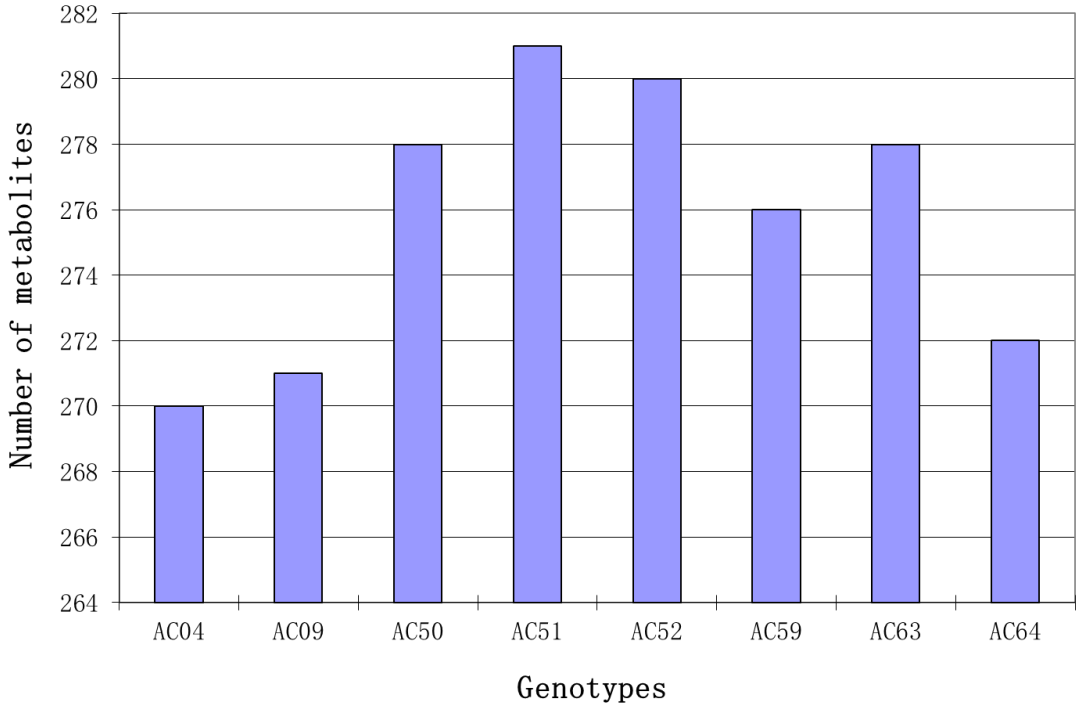

Figure 3. 2 : The number of metabolites detected in each of the eight diploid potato genotypes.

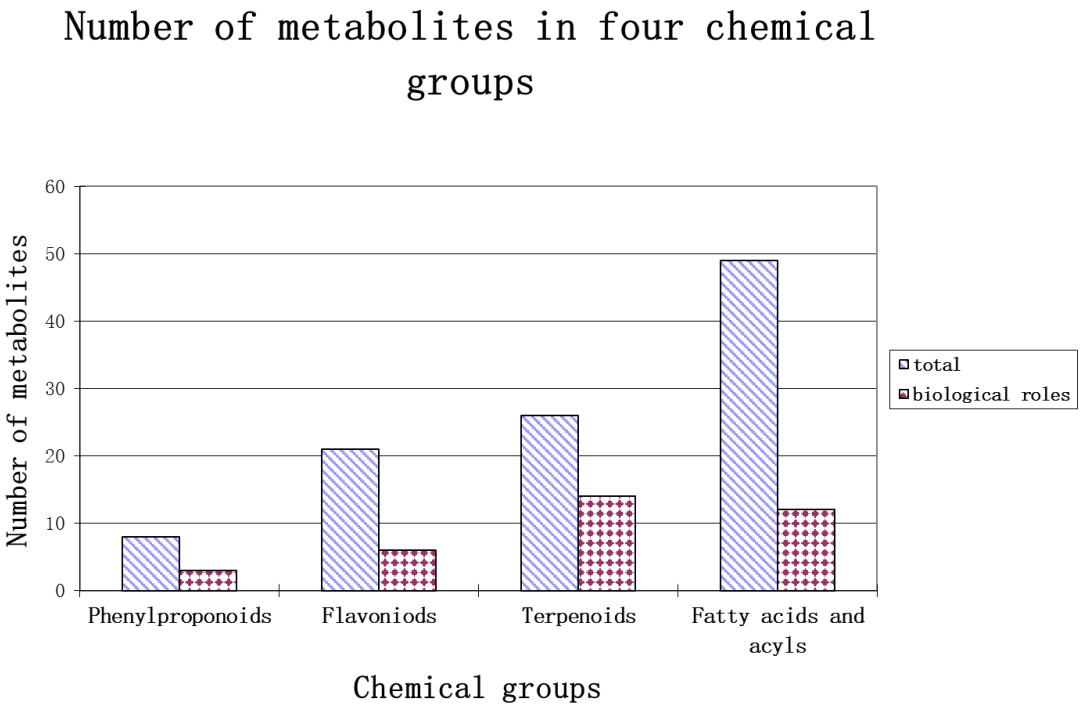

Figure 3. 3: The total number of metabolites detected and the number of metabolites which have been reported to have biological roles in four chemical groups.

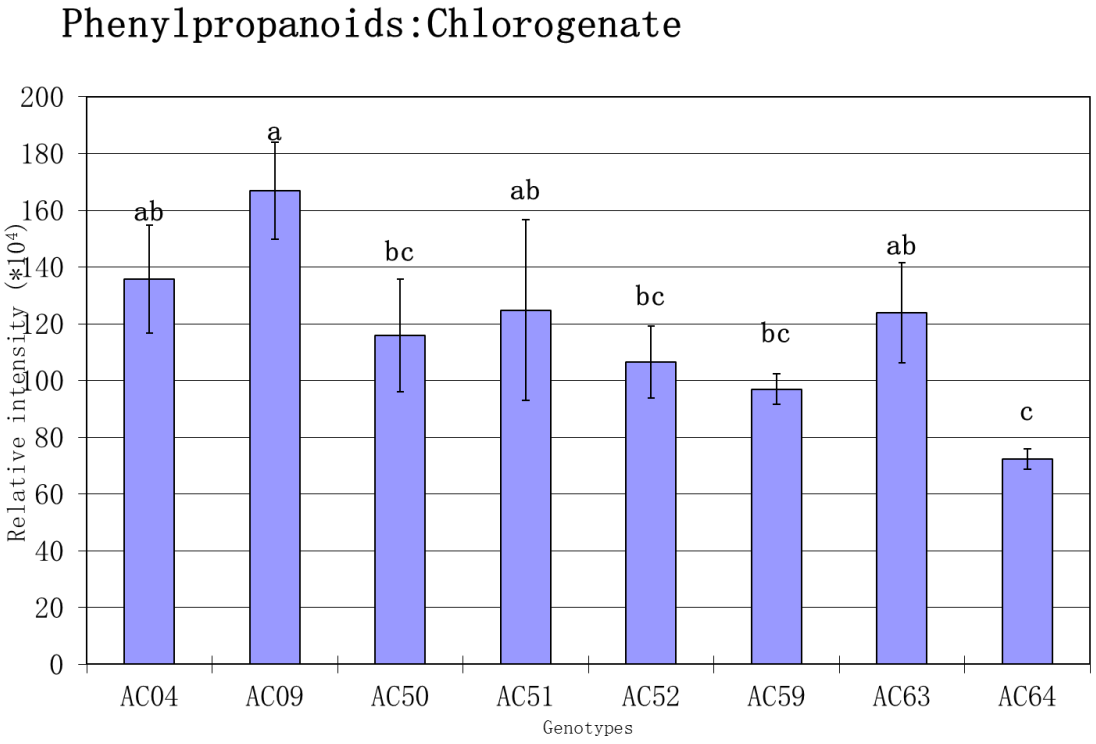

Figure 3. 4: The relative intensity of chlorogenate detected in eight diploid potato genotypes. The bar with same letters are not significant at P<0.05.

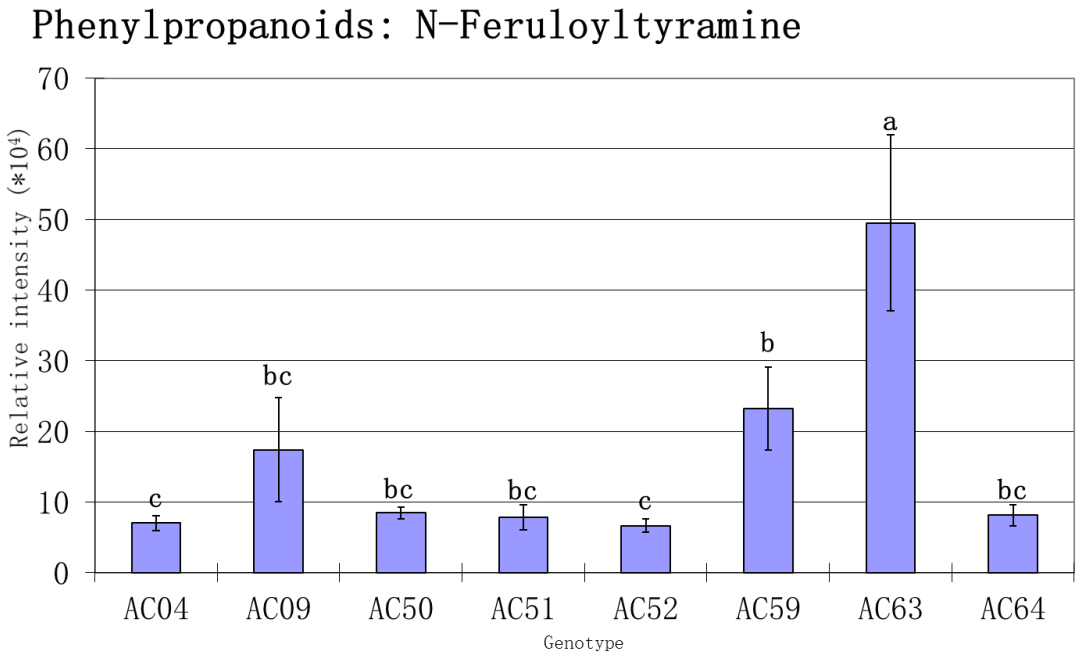

Figure 3. 5: The relative intensity of N-Feruloyltyramine detected in eight diploid potato genotypes. The bar with same letters are not significant at P<0.05.

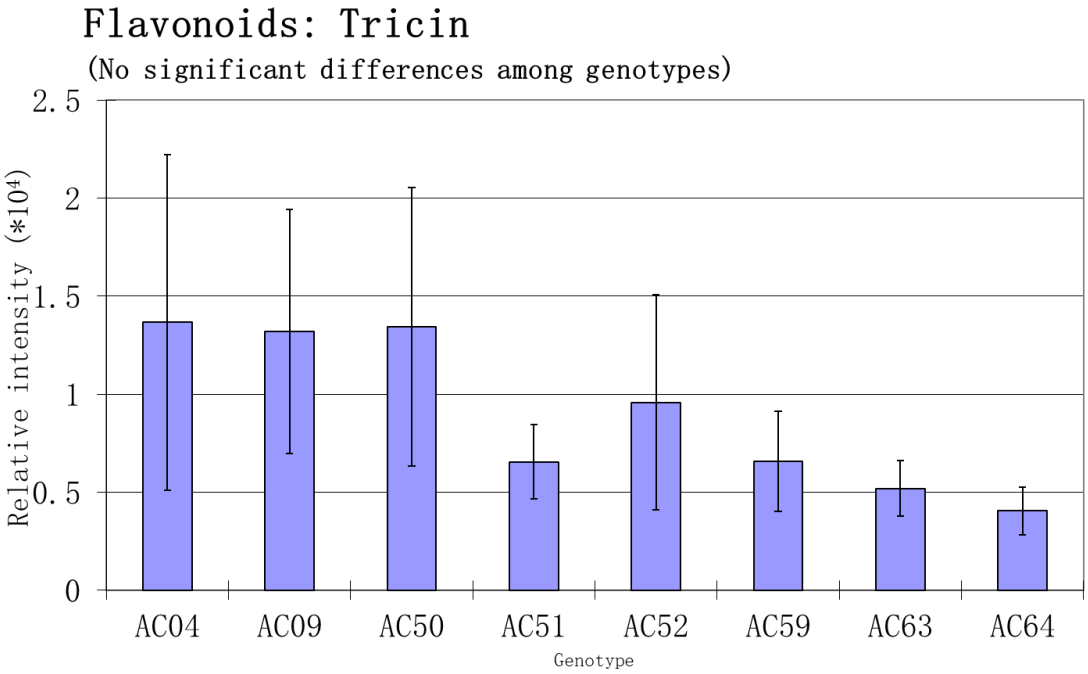

Figure 3. 6: The relative intensity of Tricin detected in eight diploid potato genotypes.

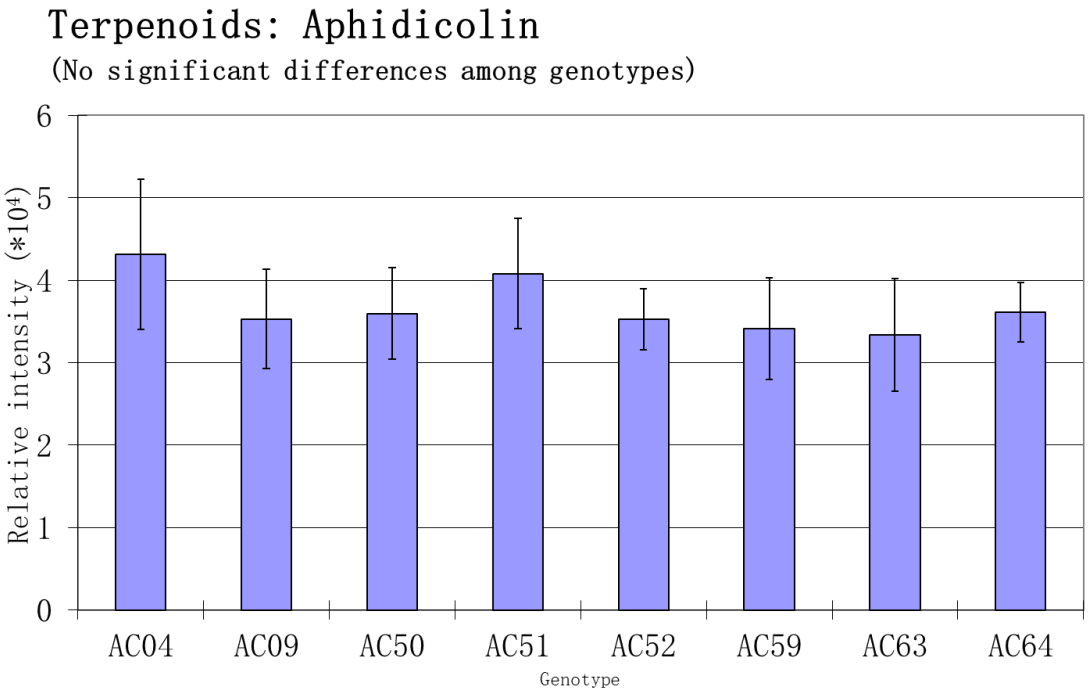

Figure 3. 7: The relative intensity of Aphidicolin detected in eight diploid potato genotypes.

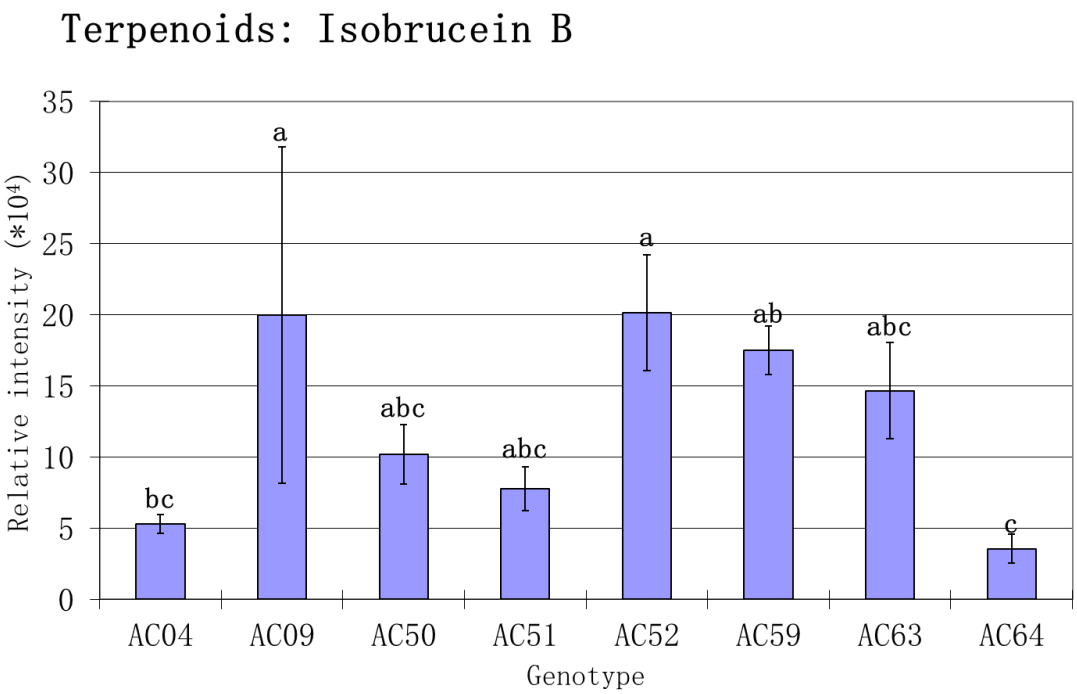

Figure 3. 8: The relative intensity of Isobrucein B detected in eight diploid potato genotypes. The bar with same letters are not significant at P<0.05.

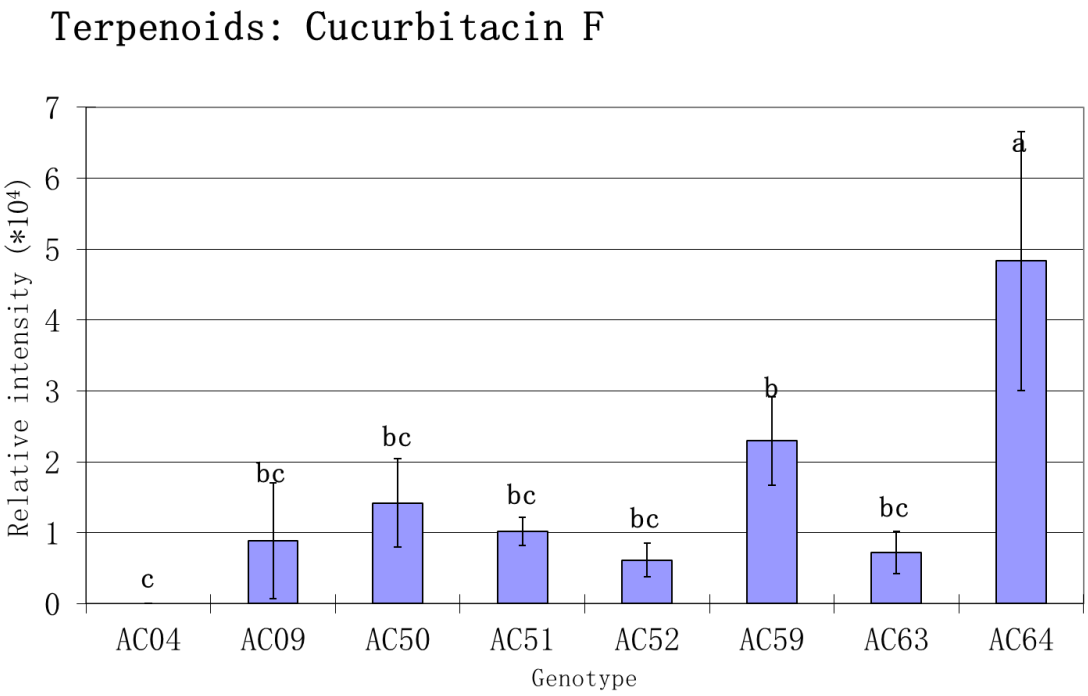

Figure 3. 9: The relative intensity of Cucurbitacin F detected in eight diploid potato genotypes. The bar with same letters are not significant at P<0.05.

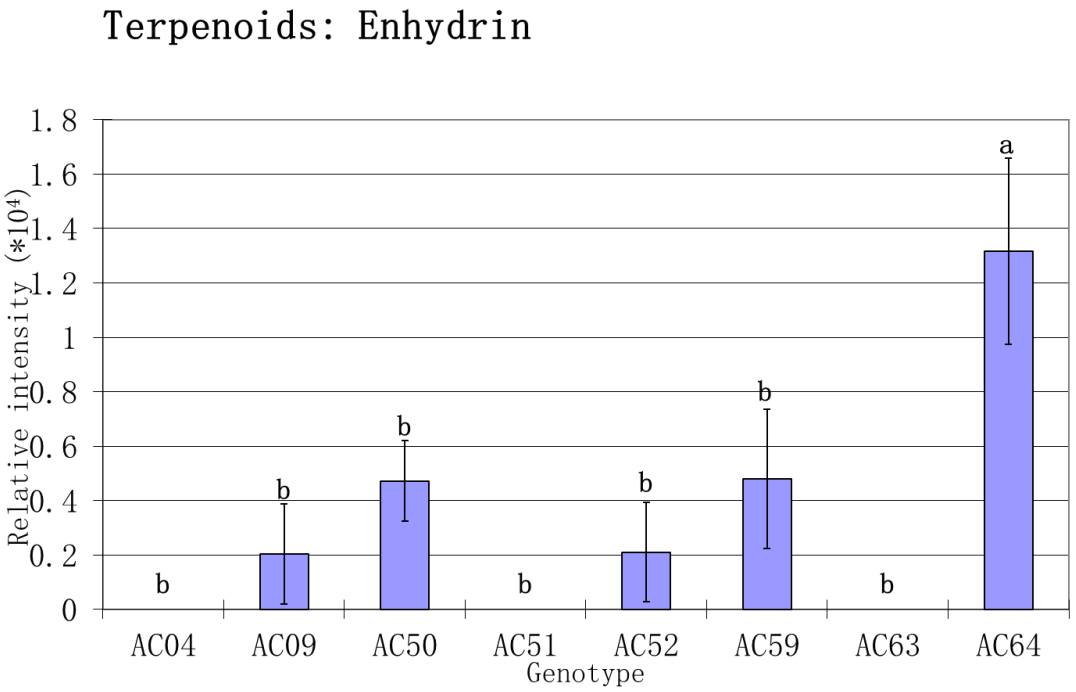

Figure 3. 10： The relative intensity of Enhydrin detected in eight diploid potato genotypes. The bar with same letters are not significant at $P<0.05$.

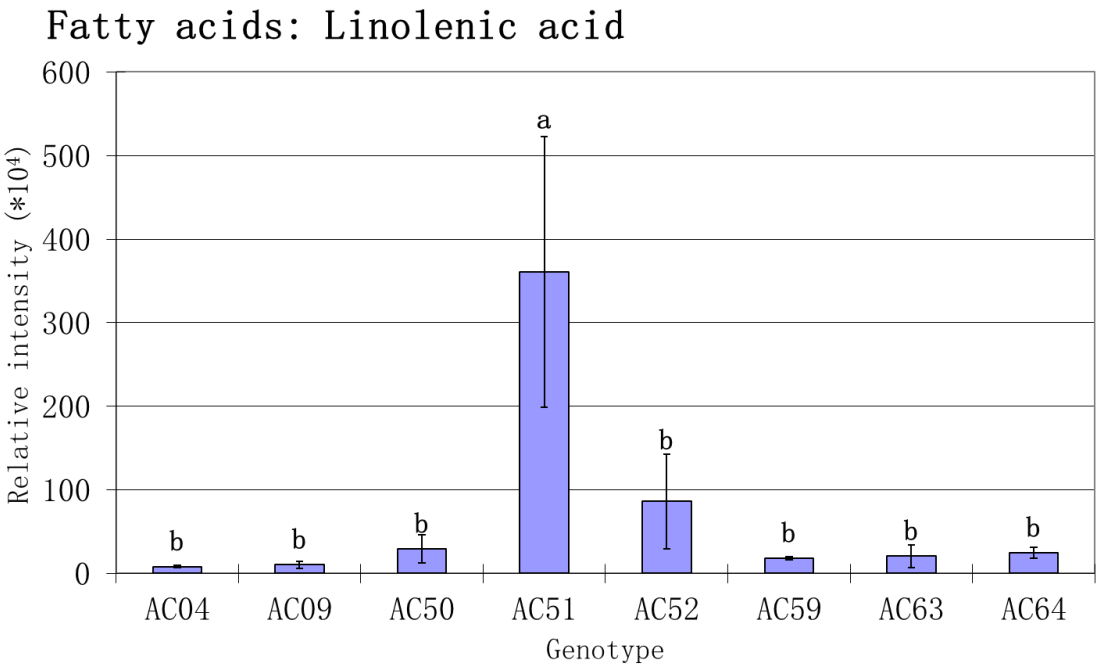

Figure 3. 11: The relative intensity of Linolenic acid detected in eight diploid potato genotypes. The bar with same letters are not significant at $P<0.05$.

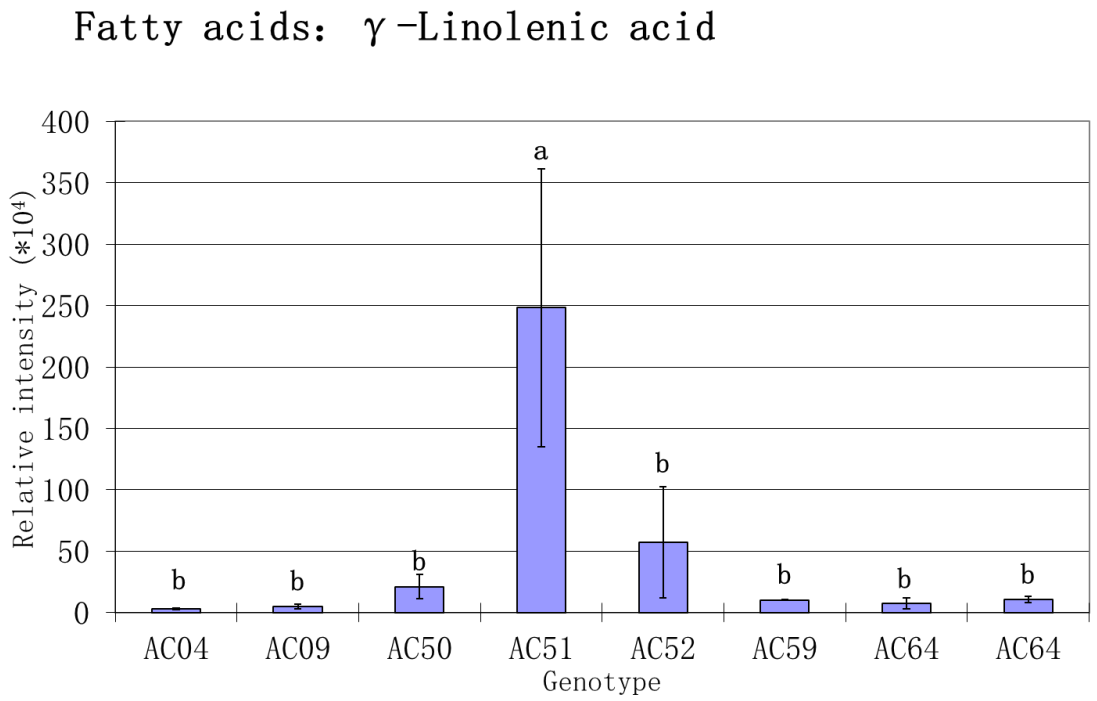

Figure 3. 12: The relative intensity of γ-Linolenic acid detected in eight diploid potato genotypes. The bar with same letters are not significant at $P<0.05$.

Chapter IV General discussion and conclusion

In this study, we have reported several functional ingredients metabolites and ranked them based on the abundance in one genotype AC04. Considering the functional value, these metabolites exhibit numerous major health benefits such as anticancer, anti-inflammatory, antimicrobial, antioxidant, and anti-HIV. The results showed that different genotypes can be explored for important metabolites which showed health benefiting properties. Specific genotypes with high abundance of functional ingredients reported here can be used in the Colombian breeding programs to increase a specific metabolite. Alternatively, similar studies can be conducted on other potato cultivars to explore other cultivars with high abundance of a given metabolite, and in turn this cultivar can be used in breeding programs to enhance the compound. Now that the potato genome has been sequenced, it is also possible to find the genes responsible for the production of these specific functional ingredients metabolites and use them to replace them in the elite cultivars, if they are polymorphic or nonfunctional (Kushalappa and Gunnaiah 2013).

Chapter V Suggestions for Future Research

This study reports only the relative abundances of these metabolites, further research is needed to determine whether those metabolites are present in significant concentrations to exert a functional effect. Besides, in order to enhance the content of the metabolites, the candidate metabolites should be mapped in the biosynthesis pathway to identify the enzymes, then they will be searched in the genomic database to identify the genes which regulate them. These genes can be transferred to the elite cultivars through molecular biology technologies to increase the amount of the desired metabolites (Kushalappa and Gunnaiah 2013).

Chapter VI Contributions to Knowledge

This is the first study that investigated the metabolites in cooked diploid potatoes from Colombia based on non-targeted metabolomics approach. This study reports several metabolites with functional ingredients values. It reported the chemical groups and the number of metabolites detected in each genotype. It also gave an idea of the ranking of abundance of the metabolites in eight genotypes. The study also identified metabolites that remained unchanged even after cooking. Finally, this study provided several functional ingredients metabolites with high fold change in certain genotypes, which can be used in Colombian potato breeding programs to improve functional ingredients in potato cultivars of the indigenous communities.

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Appendix

Table S1: The fragmentation pattern of the putatively identified functional ingredients related metabolites in potato genotypes

observed mass[c] (Da)	exact mass (Da)	Metabolites/chemical group	RT[d]	AME[e] (ppm)	Chemical formula	Observed fragmentation[b]	Database fragmentation[a]
Phenylpropanoids							
354.0962	354.0951	Chlorogenate	2.56	3.05	C16H18O9	**179.41**,183.13,**191.11**,193.19,301.98,323.28	163.03, 175.06, **179.03**, **191.65**, 337.09
313.1323	313.1314	N-Feruloyltyramine	17.17	2.85	C18H19O4	172.19,17311,183.15,184.25,185.14,186.11,186.63,200.08,212.25,241.17,251.35,256.23,269.41,282.00,**295.16,297.27**,313.25,314.24	**296.13, 298.11**
Flavonoid							
288.0624	288.0634	Dihydrokaempferol	30.34	-3.35	C15H1206	**93.01**,110.96,126.97,141.12,147.20,155.29,169.22,176.97,185.21,**195.09**,200.07,209.11,219.16,225.40,231.49,247.24,259.33,**271.22**,290.61,291.31	17.00, **93.03**, **195.03**, **271.06**
330.073	330.074	Tricin	28.99	-2.88	C17H14O7	121.80,129.32,150.16,160.30,**175.21**,181.30,193.75,202.07,209.13,222.03,260.18,285.27,**299.93**,**312.27**	15.02, 17.00, 31.01, 153.05, **177.02**, **299.06**, **313.07**, 315.05
Terpenoids							
338.244	338.2457	Aphidicolin	28.89	-4.90	C20H34O4	119.09,139.15,147.14,156.05,170.15,183.06,184.16,197.13,211.46,225.16,239.22,253.22,267.26,275.37,281.24,295.25,307.18,**321.33,337.36**,340.38	**321.24**, 323.22**, 337.24**
692.3396	692.3408	Scillaren A	13.60	-1.78	C36H52O13	439.26,468.22,**513.31**,576.80,611.78,629.18,647.34,**675.16**	163.06,179.06,309.12,325.11,367.23,383.22, **513.29**, 529.28, 597.33, **675.34**, 677.32
522.2115	522.2101	Isobrucein B	15.12	2.64	C26H34O11	180.41,226.13,253.95,341.26,365.36,405.21,423.58,433.56,462.17,**491.94**,**504.08**	**491.19**, **505.21**, 507.19, 521.20
414.1173	414.1162	Asperuloside	9.60	2.76	C18H22O11	131.32,161.10,170.99,221.08,**222.95**,229.22,231.05,235.22,245.01,253.13,265.04,**267.00**,**285.90,296.24**,299.14,308.98,311.07,317.06,328.15,341.13,342.12,352.86,355.06,369.30,371.08,385.02,387.94,396.96	56.05,61.01,104.05,121.06,136.07,146.06,148.08,193.10,202.08, **222.09**,240.07,250.09,**267.11**,**285.09,295.11**

518.3259	518.3244	Cucurbitacin F	15.47	2.91	C30H4607	147.12,152.80,164.26,171.35,186.16,213.06,244.20,259.04,261.13,277.96,279.30,309.27,315.11,316.43,331.44,351.35,**361.11**,397.33,407.01,427.09,431.31,435.58,480.28,**501.38**	157.09, **361.24**, 459.27, **501.32**, 503.30, 517.32
356.1118	356.1107	Gentiopicrin	2.64	3.20	C16H20O9	133.40,**149.15**,164.24,179.15,194.13,207.23,217.20,252.34,266.99,273.73,**281.0**7,299.10,309.47,**322.40**,341.23,356.26	43.02,67.0,85.07,136.06,145.07,**148.06**,187.10,202.11,220.12,238.13,262.13,**280.14**,**322.15**
464.1698	464.1682	Enhydrin	8.64	3.37	C23H28O10	175.37,193.08,197.05,215.06,227.02,249.16,253.07,167.19,274.97,289.10,293.12,302.96,309.25,**393.50**,403.22,421.34,446.88,**450.04**,457.16	**393.12**, **449.14**, 463.16
206.1679	206.1671	Alpha-Irone	27.05	4.01	C14H220	110.22, **163.28**, 207.33	137.13, **163.15**, 191.14
Fatty acids							
282.2566	282.2559	(9Z)-Octadecenoic acid	29.32	2.38	C18H34O2	107.17,112.06,124.99,133.10,146.04,150.12,151.08,164.31,186.25,192.21,**197.18**,**208.35**,221.04,**226.18**,**237.33**,249.34,255.06,**265.24**,282.19	101.06, 115.07, 129.09, 153.16, 167.18, 181.20, 183.14, 195.21, **197.15**, **209.23**, 211.17, 223.24, **225.18**, **237.26**, 239.20, 253.22, **265.25**, 267.23
270.2566	270.2559	Methyl palmitate	29.66	2.52	C17H34O2	147.02,159.02,**169.06**,201.16,**211.35**,212.70,**213.43**,**227.19**,229.15,230.95,255.04	141.16, 143.11, 155.18, 157.12, **169.20**, 171.14, 183.21, 185.15, 197.23, 199.17, **211.24**, **213.19**, **227.20**
280.241	280.2402	Linoleic acid	28.36	2.72	C18H32O2	98.94,111.12,117.23,147.23,154.95,173.76,183.40,199.13,211.45,225.07,**237.22**,**251.45**,**263.39**,**264.48**,276.99,281.36282.17	101.06, 129.09, 151.15, 179.18, 207.21, 209.15, 221.23, 223.17, 235.24, **237.18**, **251.20**, **263.24**, **265.22**
278.225405	278.2246	Γ-Linolenic acid	27.47	2.89	C18H30O2	80.12,82.08,**97.10**,**111.32**,123.49,**128.19**,**141.32**,147.38,158.97,**167.08**,**181.23**,193.14,197.23,**205.43**,211.19,224.24,**235.21**,243.19,251.20	87.04, **97.10**, 101.06, **111.12**, **127.08**, 137.13, **141.09**, 151.15, **167.11**, 177.10, **181.12**, 191.18, **205.20**, 207.14, 219.21, 221.15, 233.23, **235.16**, 249.19, 261.22, 263.20
200.1784	200.1776	Dodecanoic acid	24.68	4.22	C12H24O2	**74.10**,**86.16**,97.32,**116.12**,121.86,**130.18**,**141.57**,142.16,144.11,**157.16**,159.14,160.04,167.29,173.15,**185.19**,186.15,188.10,203.11	71.09, **73.03**, **85.10**, 87.04, 99.12, 101.06, 113.13, **115.07**, 127.15, **129.09**, **141.16**, 143.10, 155.18, **157.12**, 171.14, 183.17, **185.15**,
158.1315	158.1307	Nonanoic acid	19.01	4.99	C9H18O2	63.05,**71.08,73.10**,79.23,82.27,91.12,**100.03**,103.18,118.18,122.24,134.24,137.22,147.08,147.95,164.12	57.07, 59.01, **71.08, 73.03**, 85.10, 87.04, **99.12**, 101.06, 113.13, 115.08, 129.09, 141.12, 143.10

256.2407	256.2402	Hexadecanoic acid	28.89	2.14	C16H32O2	136.40,**157.95**,175.06,**199.03**,**238.49**,256.51	85.10, 99.11, 101.06, 115.08, 127.15, 129.09, 143.11, 155.18, **157.12**, 169.20, 171.14, 183.21, 185.15, 197.22, **199.17**, 211.24, 213.19, 227.20, **239.24**, 241.22
188.1057	188.1049	Azelaic acid	1.77	4.11	C9H16O4	**59.12**,67.30,71.10,83.05,85.12,**87.08**,93.12,99.10,109.14,111.01 ,**115.13**,125.23,127.10,**129.12**,137.08,**143.08**,147.17,155.10	**59.01**, 73.03, **87.04**, 101.06, **115.08**, **129.09**, **143.11**, 171.10
186.1628	186.162	Undecanoic acid	23.70	4.30	C11H22O2	**57.20**,**87.94**,136.29,137.11,**157.07**,162.16,162.98	**57.07**, 71.09, 85.10, **87.04**, 99.11, 101.06, 113.13, 115.08, 127.15, 129.09, 141.16, 143.11, **157.12**, 169.16, 171.14

[a]Database fragmentations were obtained through massbank database.

[b]Observed fragmentation patterns were obtained through LC-LTQ-Orbitrap.

[c]Mass (m/z) observed corrected for H (1.0078) as it was analyzed in negative mode.

[d]RT=Retention time

[e]AME= Accurate mass error (ppm) was calculated using the formula [(observed mass-exact mass)/exact mass]*1

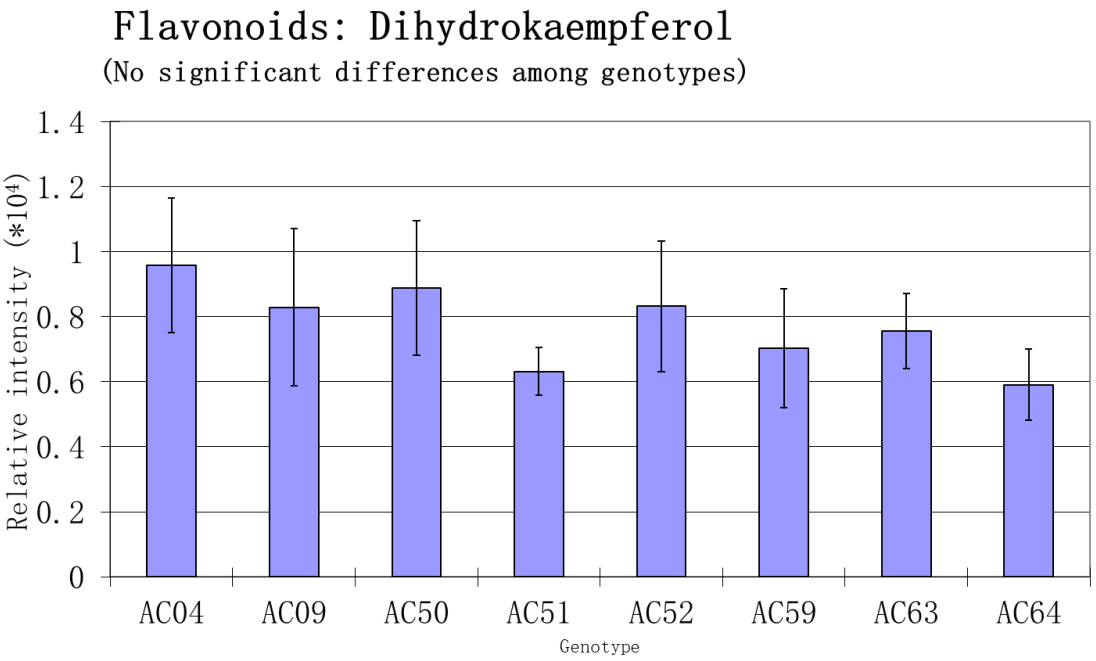

Figure S1: The relative intensity of Dihydrokaempferol detected in eight diploid potato genotypes.

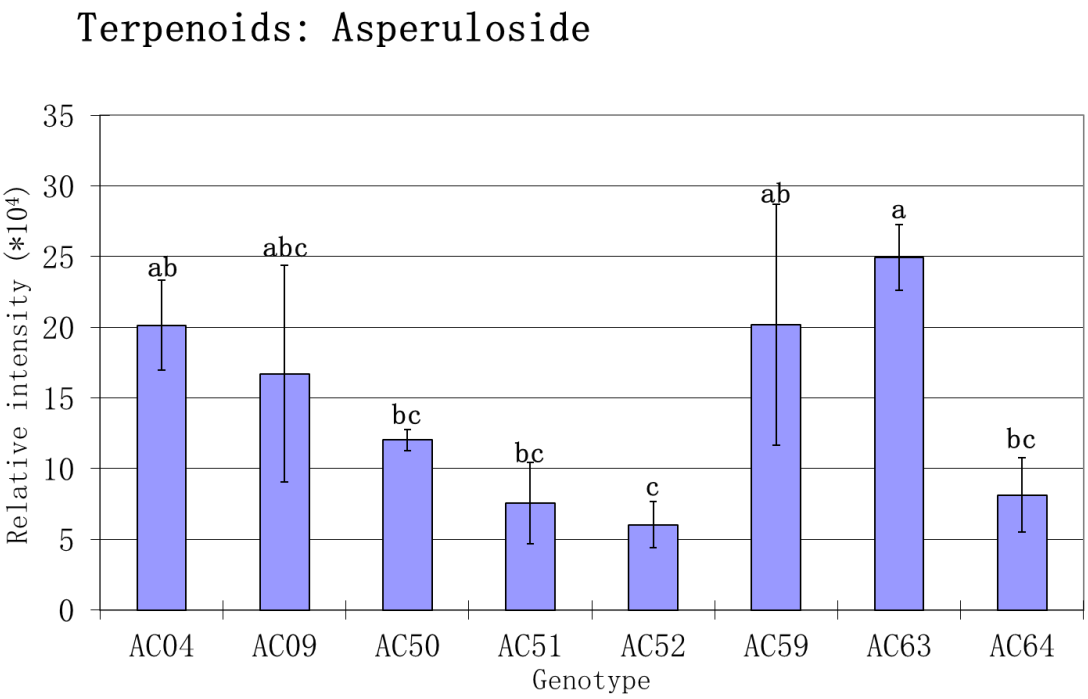

Figure S2: The relative intensity of Asperuloside detected in eight diploid potato genotypes. The bar with same letters are not significant at P<0.05.

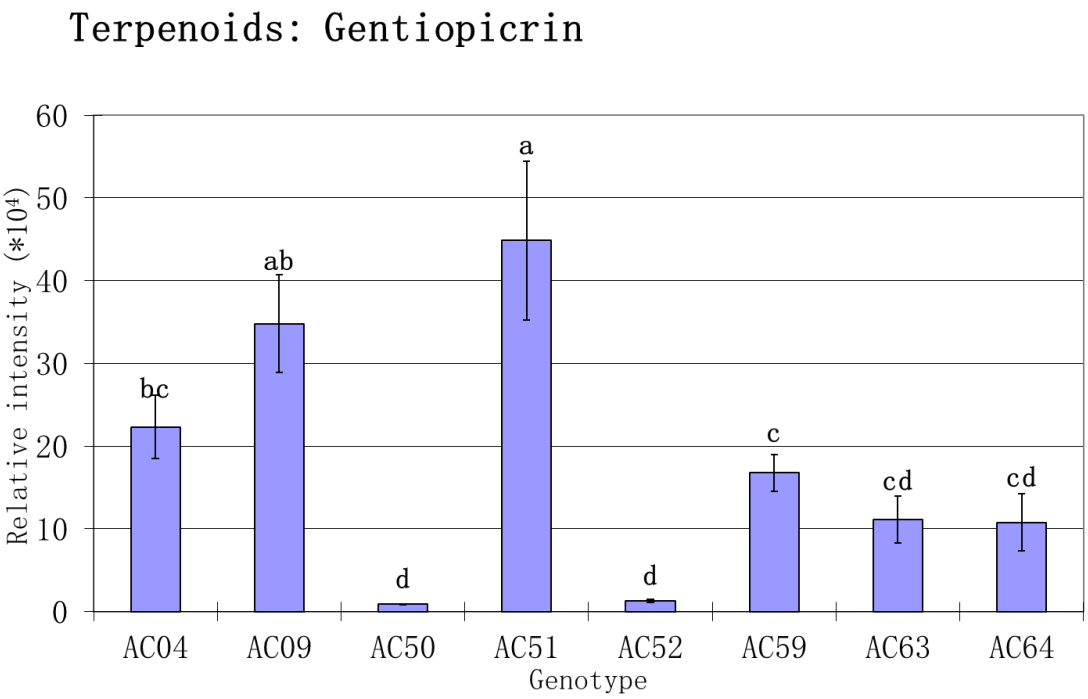

Figure S3: The relative intensity of Gentiopicrin detected in eight diploid potato genotypes. The bar with same letters are not significant at P<0.05.

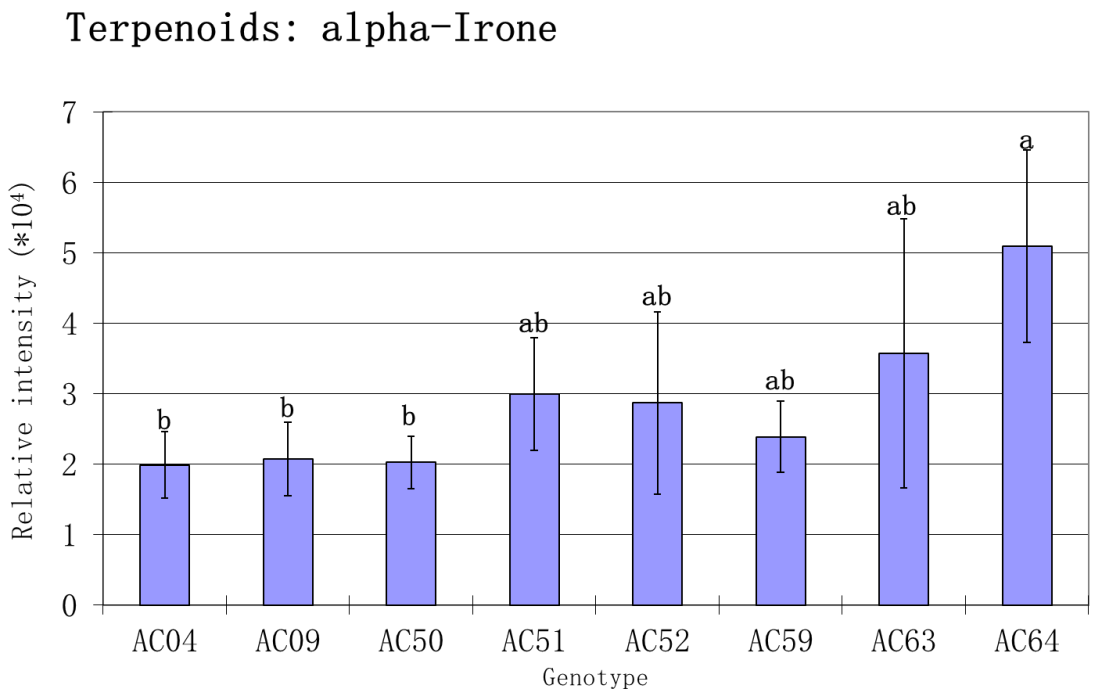

Figure S4: The relative intensity of alpha-Irone detected in eight diploid potato genotypes. The bar with same letters are not significant at $P<0.05$.

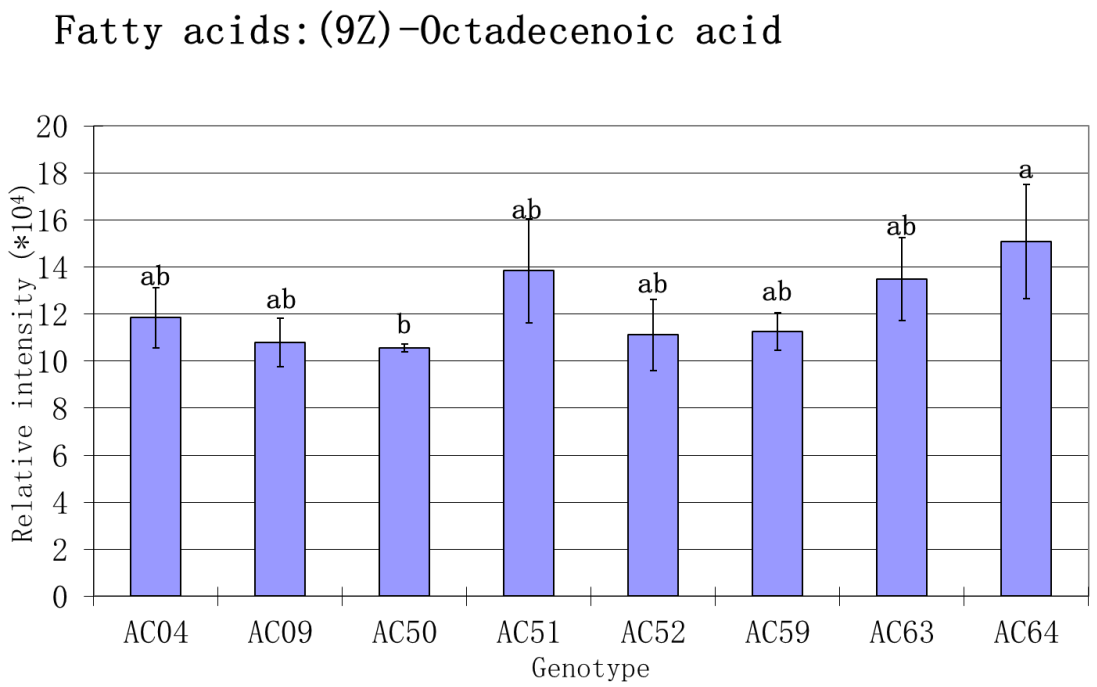

Figure S5: The relative intensity of (9Z)-Octadecenoic acid detected in eight diploid potato genotypes. The bar with same letters are not significant at $P<0.05$.

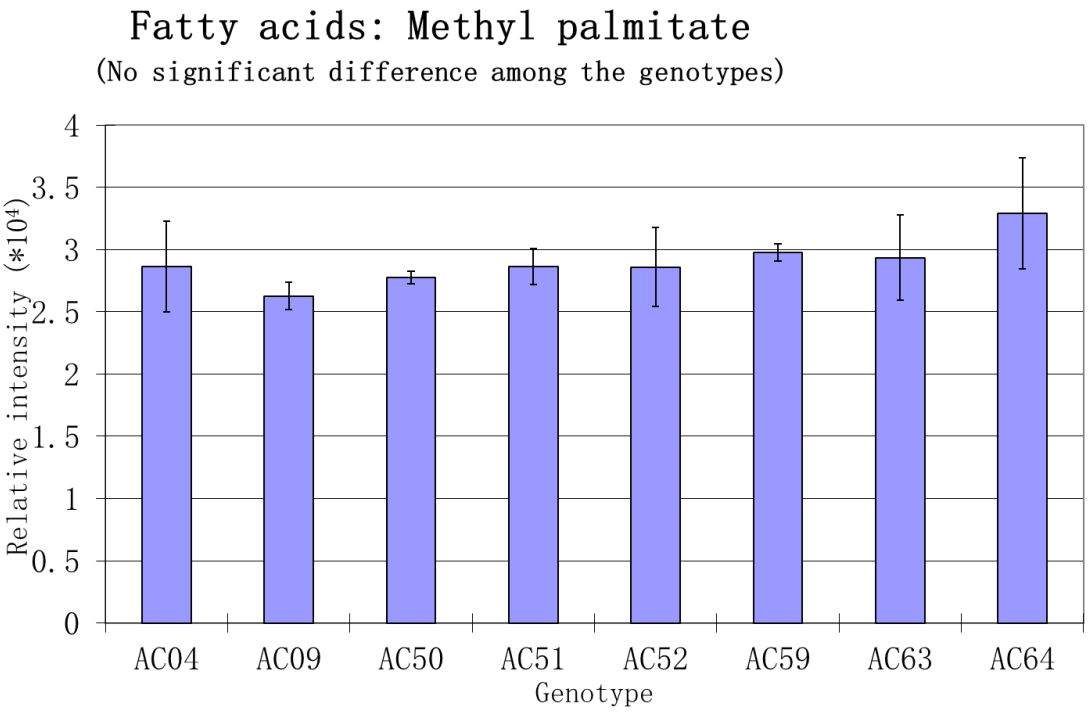

Figure S6: The relative intensity of Methyl palmitate detected in eight diploid potato genotypes.

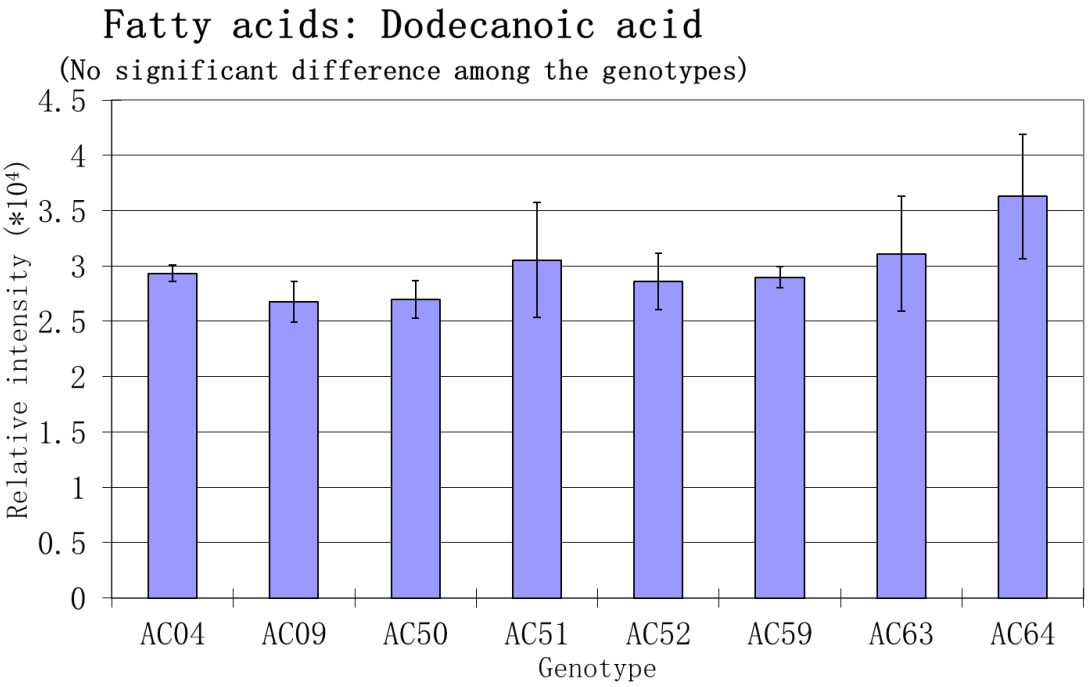

Figure S7: The relative intensity of Dodecanoic acid detected in eight diploid potato genotypes.

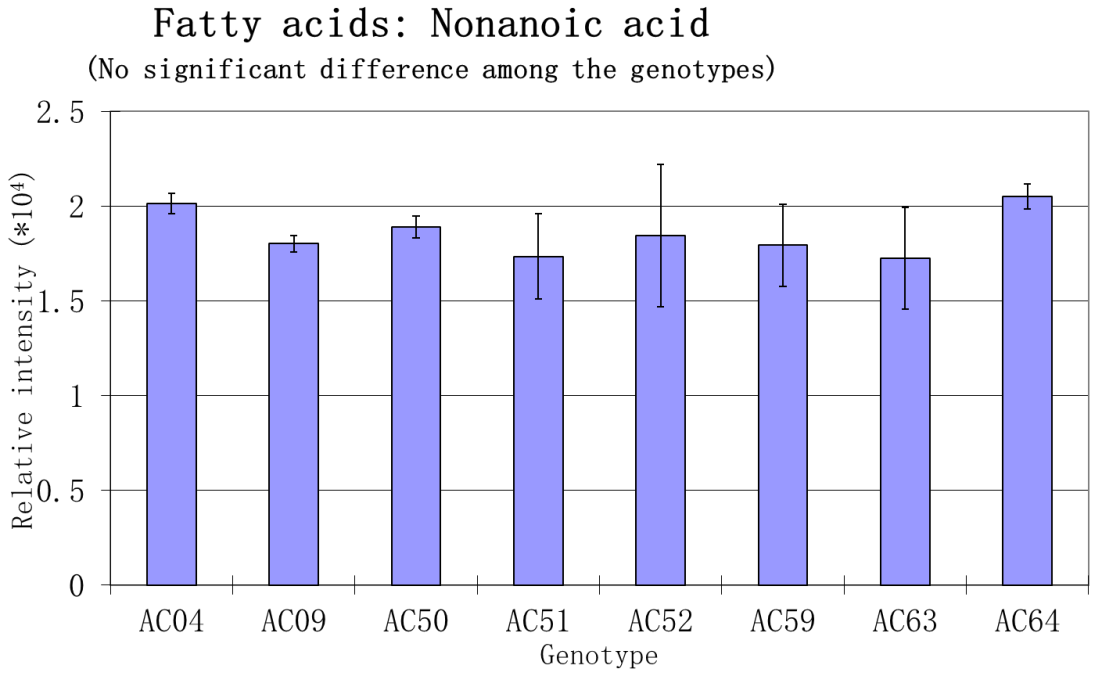

Figure S8: The relative intensity of Nonanoic acid detected in eight diploid potato genotypes.

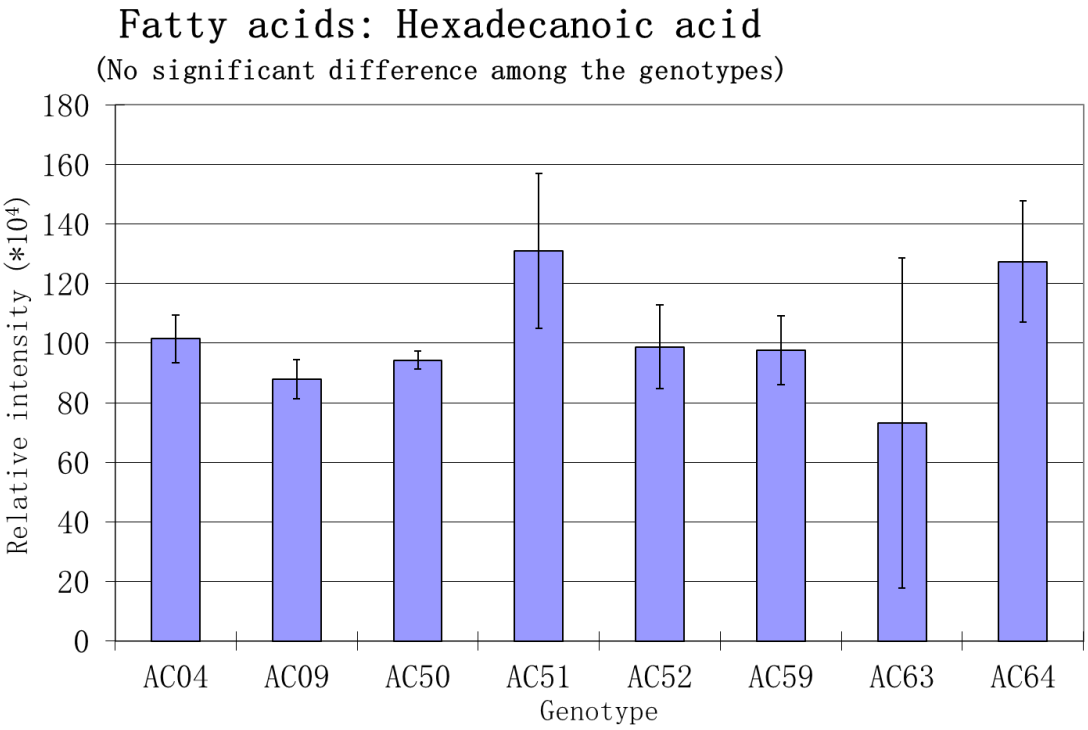

Figure S9: The relative intensity of Hexadecanoic acid detected in eight diploid potato genotypes.

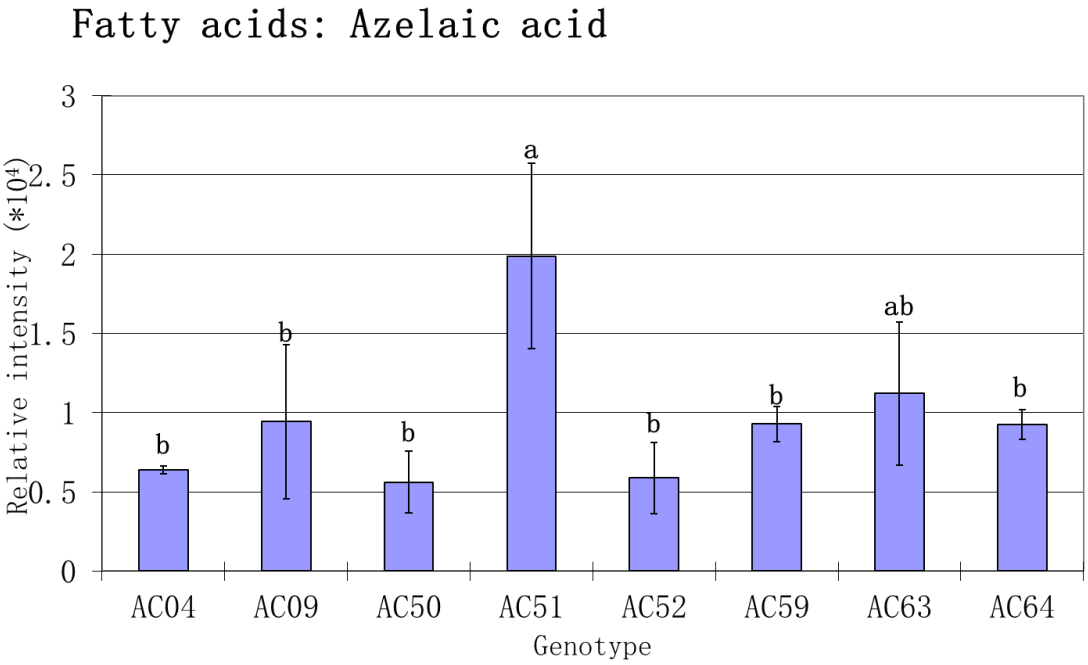

Figure S10: The relative intensity of Azelaic acid detected in eight diploid potato genotypes.

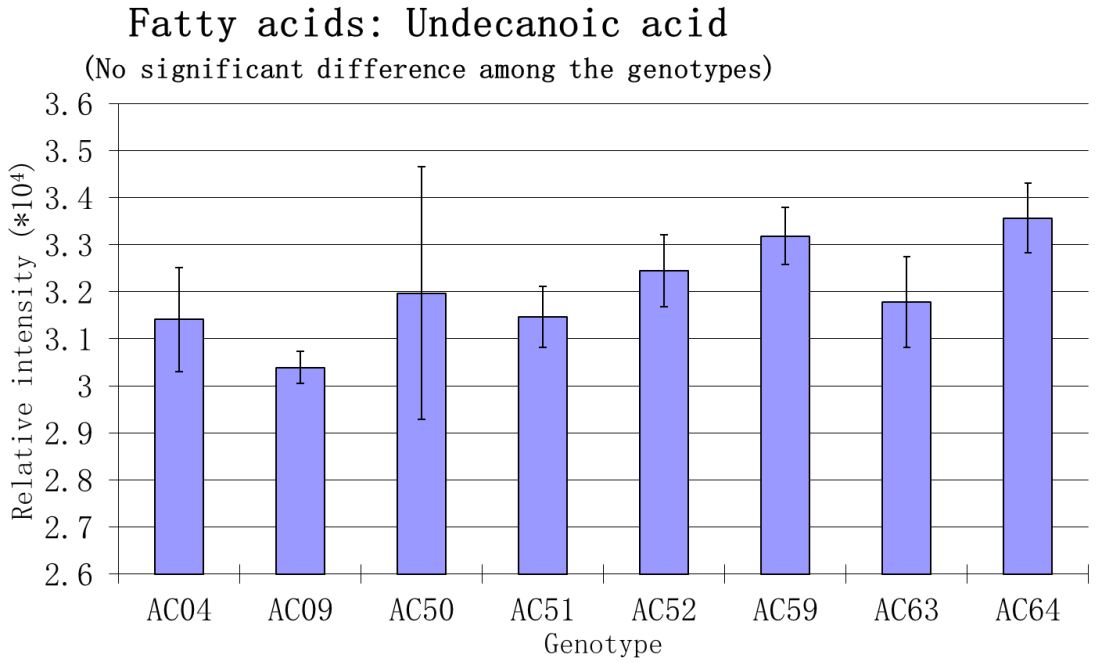

Figure S11: The relative intensity of Undecanoic acid detected in eight diploid potato genotypes.