Marshall Plan Scholarship – report

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Title:

Identification and characterization of phytochemicals with potential human health promoting effects

Subtitle:

Apple juice as a superior source of promising phytochemicals

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Summary

Increasingly popular discussion topics revolve around the positive effects and potential use of phytochemicals to prevent diseases due to their antioxidant potential. At present, there are many different ways to characterize the molecular action of phytochemicals within living cells. Methods that determine the bio-availability of selected active ingredients and fluorescent microscopy based assays are of particular interest because they are used to analyze the molecular effects of such substances in human cells. It is also important to study the effects of phytochemicals on protein-protein interactions or on the cellular distribution of proteins. Characterizing such interactions can provide insight on how specific compounds work as well as to help provide a better understanding of integral processes such as gene expression, intercellular communication, nutrient uptake and cell growth.

The benifit of fluorescent microscopy is that it is commonly used to identify small organisms, such as microbes. Analyzing small organims such as microbes with fluorescent microscopy is very useful because it can be used to obtain a 3-Dimensional (3-D) story for researchers at or near the cell's surface. It utilizes fluorescent probes which attach to targeted structures. These structures are then highlighted by light wave lengths, which enable scientists to picture targets.

Another important question which is of special interest is the bioavalibity of phytochemicals. Bioavailablity studies help characterize and define adsorption levels of

drugs and active ingredients from plants in humans. It is usually measured by a percentage which defines the fraction at which the human body is able to access a drug dose. There are physical factors that can inhibit or increase adsorption rates into the human system. Food, drugs and metabolism are a few common examples of factors that can affect the rate of drug adsorption. Overall, bioavailability studies can help increase understanding of specific drugs or compounds and how they can be utilized for maximum effectiveness.

In order to utilize the aforementioned methods, different extracts and juices containing phytochemicals must be prepared. It is important to note that the plant extracts must be of high quality in order to be effectively and accurately characterized. This includes storage, preperation and handling of samples. This is one of the major questions that has been addressed within this project: apples are a superior source of phytochemicals, but their abundance is highly dependent on the apple variety. Therefore, the phytochemical composition of almost 80 apple varieties organically grown in Lower Austria have been analyzed, using various methodologies, including HPLC, fluorometric, and binding assays.

High Performance Liquid Chromatography (HPLC) was used to identify and quantify components in the apple juice mixture. It is a chromatographic technique that uses separation methods to extract differnet substances.

Purified phytochemicals or mixtures containing these substances that have been analyzed will be used in future studies to address different biological questions of key relevance. The first question targets the investigation and identification of insulin mimetic substances. Insulin mimetic substances are constituients that have the potential to resemble and function similarly to the peptide protein, insulin. Defining insulin mimetic substances is important in diabetes associated research because insulin provides the body with a means to absorb glucose. If the body becomes resistant to insulin, blood glucose levels can build up in the body in spite of high levels of insulin. This is an indcation of type 2 Diabetes. Likewise, insulin resistance is a symptom of diabetes, making it integral to identify and treat said symptoms. Recently a method has been developed that allows for a fast and quantitative characterization of active ingredients, that increase the uptake of glucose and thus lower the blood-glucose levels independent of insulin. Some of these known active ingreditents include phytochemicals. This approach is based on TIRF microscopy and the assay is utlized to identify new and better characterize known insulin mimetic substances. The described project has already started and the apple phytochemicals identified within this project will be of key importance to identify novel insulin mimetic substances isolated from apples.

Of equal importance is the identification of apple derived polyphenols which modulatethe activity of the Epidermal growth factor receptor (EGFR). The EGFR is a cell surface receptor and a known oncogene in humans. Its overexpression may lead to uncontrolled cell proliferation, which is tied to cancer.

Taken together, results obtained from the experiments performed within in this project successfully provided a basis to investigate the role of apple derived phytochemicals in different biological processes.

Introduction

In a perfect world, everything the human population consumed would serve a positive health function. It's no secret, however, that targeting naturally grown fruits and vegetables helps the human population take one step closer to achieving a powerful diet. Currently, the most popular fruits and vegetables include berries, apples, bananas, potatoes and tomatoes. Understanding the phytochemicals and other health promoting agents within these foods can help advance our knowledge of nutrition related to disease prevention.

In Austria, apples are the most frequently cultivated fruit with more than 170,000 t harvested in 2013 (Statistic Austria). However, two thirds of the total apple harvest of 2012 was composed of only four varieties (25% Golden Delicious, 21% Gala, 10 % each Idared and Jonagold). Due to the fact that Golden Delicious, Gala, Idared and Jonagold dominate the apple market, many old, less common, varieties are on the verge of extinction. Many of the old varieties that are on the verge of extinction do not comply or fully meet current marketing schemes and consumer demands. Common consumer demands include size, colour, texture and taste. Unfortunately, these demands do not reflect the overall health rating of apple cultivars. In order to preserve and market the apples which possess high health ratings, detailed knowledge of the composition and content of beneficial compounds is required. Such knowledge is only available for a fraction of cultivars (Lee 2003, Harker 2003, Ceymann 2012, Kahle 2005, Andre 2012, Jakobek 2013, Eisele 2005). Naturally, the more rare the cultivar, the less likely it is researched and documented. This lack of information is highlighted when examining rare Austrian cultivars.

When examining overall apple health ratings- it is important to understand exactly why an apple a day can keep the doctor away. In the context of this study, the beneficial health effects of an apple diet have been attributed to the secondary plant metabolites designated as phenolic compounds. Secondary plant metabolites include terpenes, phenolics and nitrogen-containing compounds and are classified as any chemical produced by a plant that does not serve a fundamental role in the growth and/or survival of the plant.

Phenolic compounds are known for their antioxidant potential and can thus counteract detrimental effects of reactive oxygen species and free radicals (Lee 2003). Other reported effects include lowering risks of cancer and cardiovascular diseases (Boyer 2004, Habauzit 2012, Le Marchand 2000, Manach 2005, Bonita 2007). A study showed an inverse correlation to coronary heart disease in elder males to their consumption of flavonoids (Hertog, 1993). Also discovered was a positive correlation between polyphenols and decreased risk for type II diabetes (Barbosa 2010, Bidel 2008).

Polyphenolic compounds in apples are mainly flavan-3-ols, flavonols, dihydrochalcones and hydroxycinnamic acids (Lanzerstorfer, 2014, Eisele 2005). Only low molecular weight (mostly monomeric) polyphenols are water soluble and can be easily absorbed in the gut (Manach 2005) whereas polymeric compounds need to be degraded by the colon microflora in order to be absorbed to be absorbed (Monagas 2010).

The majority of published studies investigating polyphenols in apples did not analyse pure juice but rather analysed extracted polyphenols from the flesh (Tsao 2003) or peel (Denis 2013). Other studies utilized puree (Wojdylo 2008) and juices (Kahle 2005) for analysis. In contrast to these studies, this study analysed freshly pressed juices without any additional treatment. In doing so, this study provides a more accurate representation of the polyphenols present when apples are consumed by the human population.

The aim of this study was to characterize a total of 76 apple varieties cultivated in Austria by using freshly pressed juice from each variety. The individual polyphenol fingerprint was determined by HPLC measurements. In addition total phenolic content, antioxidant capacity and selected anions and cations were quantitated. Of these 76 varieties 19 have been recently described by us (Lanzerstorfer, 2014). Consequently, we were able to compare the composition of these varieties when grown in two geographically and climatically different regions of Austria.

Materials and Methods

Two studies are presented in this research. Although many of the material and methods are similar, they display slight modifications. Therefore, both material and methods are presented below.

Study 1: Bioanalytical Characterization of Apple Juice from 88 Grafted and Non-Grafted Apple Varieties Grown in Upper Austria

Materials and Reagents. (+)-catechin, α-amylase from porcine pancreas, AAPH (2,2´-azobis(amidinopropane) dihydrochloride), ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), acetonitrile, AG1478, bovine catalase, epidermal growth factor (EGF), fluorescein sodium salt, Folin-Ciocalteu reagent, in-vitro toxicology assay kit, methanosulfonic acid, phenolphthalein, potassium persulfate, potassium phosphate, nitric acid, rosmarinic acid, sodium bicarbonate, sodium hydroxide, trifluoroacetic acid (TFA) and Trolox [(±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] were purchased from Sigma Aldrich (Taufkirchen, Germany). Standards for K^+, Mg^{2+} and Ca^{2+} (Certipur, cation multi-element standard III) and antimony tartrate solution were from Merck-Millipore (Vienna, Austria). Potassium dihydrogen orthophosphate, ascorbic acid, methanol and ammonium molybdate were purchased from VWR (Vienna, Austria). Sulfuric acid was obtained from Fisher Scientific (Vienna, Austria). Chlorogenic acid, epicatechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate, procyanidin

B1, procyanidin B2 and phloridzin were purchased from Extrasynthese (Genay, France). 96-well plates were from Greiner Bio-One (Kremsmünster, Austria). A protein phosphorylation analysis kit termed “cell-based Delfia assay” including an Europium-labeled anti-phosphotyrosine-antibody was obtained from Perkin Elmer (Rodgau, Germany). The Syto62 cell stain reagent was from Life Technologies (Darmstadt, Germany).

Apple Variety Selection and Preparation. All apple varieties were grown under organic conditions in the Eferding region of Upper Austria. This region termed Eferdinger Becken is a plain landscape area near the Danube River and has the most moderate climate in Upper Austria. The soil in this region consists mainly of loose or clayey sediments and is low or free of lime. The apples were collected in September and October 2010 and immediately processed to apple juice. Apples were washed and the juice was pressed out using a conventional juice maker (Kenwood JE 850 XXL). At least 10 apples harvested from three to five individual trees were processed to generate a combined juice for each variety to account for differences within apples from the same variety. The samples were stored at <-60 °C without addition of ascorbic acid. Analyzed apple varieties are shown in **Table 1**.

Table 1: 88 apple varieties under study including 27 apple varieties, which were grafted between the years 2003 and 2008 on two individual trees termed “F“ and “L“.

1	Alkmene	23	Hauxapfel	45	Roter Griesapfel	L224	London Pepping
2	Ananasrenette	24	Herrenapfel	46	Roter James Grieve	L220	Magna Super
3	Berneder	25	Jonathan	47	Roter Passamaner	L203	Mostzigeuner
4	Bismarck	26	Kammerapfel	48	Roter Stettinger	L209	Rajka
5	Boikenapfel	27	Kanada Renette	49	Samareiner Rosmarien	L200	Rheinischer Winterrambour
6	Carpetin	28	Kleiner Feiner	50	Schieblers Taubenapfel	L226	Ribston Pepping
7	Champagner Renette	29	Lesans Kalvill	51	Schmidberger Renette	L217	Roter Herbstkalvill
8	Damason Renette	30	Liberty	52	Schöner von Wiltshire	L207	Seeländer Reinette
9	Deans Küchenapfel	31	Maschanzker	53	Sommerrambour	L202	Zuccalmanglios Renette
10	Dülmäner Rosenapfel	32	Odenwälder	54	Spitzapfel	F213	Christkindler
11	Fasslapfel	33	Pilot	55	Spitzling	F221	Discovery
12	Florianer Rosmarin	34	Pinova	56	Steirischer Maschanzker	F218	Florina
13	Geheimrat Oldenburg	35	Piros	57	Weißer Passamaner	F215	Freyperg
14	Gelber Bellefleur	36	Plankenapfel	58	Weißer Winter-Taffetapfel	F225	Grüter Edelapfel
15	Gelber Edelapfel	37	Pom. Kongress	59	Winter-Goldparmäne	F223	Roter von Siemonffi
16	Glasapfel	38	Prinzenapfel	60	Zabergau Renette	F204	Royal Gala
17	Glockenapfel	39	Relinda	61	Zuccalmaglios Renette	F222	Sponheimer Flurapfel
18	Goldrenette	40	Retina	L21	Berlepsch	F21	Stäubli 2

	Freiherr von Berlepsch			4		2	
19	Graue Herbstrenette	41	Rewena	L205	Blenheim	F216	Topaz
20	Grüner Boskoop	42	Rheinischer Krummstiel	L201	Dr. Seeligs Orangenpepping	F219	Wachsrenette
21	Harberts Renette	43	Riesenboikenapfel	L211	Gewürzluiken	F210	Weißer Winterkalvill
22	Hausapfel	44	Roter Boskoop	L206	Hallauer Maienapfel	F208	Winterzitrone

Total Phenolic Content (TPC). Total phenolic content was determined using Folin-Ciocalteu reagent as described previously with small modifications (Ainsworth, 2007). In short, apple juice was centrifuged at 10,000 rpm for 10 min and only the supernatant was used for total phenolic quantitation, since the cloudiness of apple juice has been reported to influence the obtained TPC values (Huemmer, 2008). Deionized water (1.4 mL) was mixed with 16.7 µL apple juice supernatant and 83.3 µL of Folin-Ciocalteu reagent. The mixture was allowed to stand for 3-6 min at room temperature followed by addition of 167 µL sodium bicarbonate solutions (200 g/L). After 70-75 min incubation at room temperature in the dark absorbance was measured at 750 nm. Total phenolic content was expressed as (+)-catechin equivalents in mg/L apple juice. Each juice was measured in triplicates.

Oxygen Radical Absorbance Capacity (ORAC) Measurements

The ORAC assay was performed as described previously with slight modifications (Re, 1999). Apple juices were centrifuged (5 min; 10,000 rpm) prior to measurement. Supernatant was further diluted (1:200) with phosphate buffer (10 mM, pH 7.4). In short, 150 µL of fluorescein (10 nM) was pipetted into each well and 25 µL of the standard (Trolox) or diluted apple juice was added. The plate was incubated at 37 °C for 30 min in the dark followed by addition of 25 µL AAPH solution (240 mM) per well. The decrease in fluorescence of fluorescein was determined by collecting readings at excitation of 485 nm and emission of 520 nm every minute for 90 min on a plate reader (POLARstar omega, BMG LABTECH, Ortenberg, Germany). The ORAC value was calculated using the ORAC plugin of the Omega MARS plate reader software. Each juice was measured in triplicates.

Mineral Nutrients, Phosphate and Trace Elements. The minerals K^+, Mg^{2+} and Ca^{2+} were quantitated by ion chromatography (Dionex ICS1000, Thermo Fisher Scientific, Vienna, Austria). An Ionpac CS 12A 4 mm column was used for the separation of the different cations. The mobile phase consisted of 20 mM methanosulfonic acid, the flow rate was 1 mL/min, and sample injection volume was 25 µL.

Phosphate concentrations were measured using a phosphomolybdate method (Huang, 2002). The apple juices were passed through a 0.45 µm filter. 500 µL apple juice, 2 mL ddH_2O water, 100 µL ascorbic acid solution (10 g ascorbic acid in 100 mL ddH_2O) and

200 µL ammonium molybdate solution were mixed and filled up to 5 mL with ddH_2O. As a phosphate standard, potassium dihydrogen orthophosphate was used. Absorbance was measured at 880 nm.

The trace elements Mn^{2+}, Cu^{2+} and Fe^{2+} were quantitated by inductively coupled plasma mass spectrometry (ICP-MS). Prior the measurement 1 mL apple juice was mixed with 3 mL of HNO_3 and microwave-digested using the MLS Ultraclave-IV. The resulting clear solution was diluted 1:10 with ddH_2O. ICP-MS measurements were performed using an Agilent ICP-MS 7500 cx spectrometer (Agilent Technologies, Santa Clara, USA) operated in He mode (Mn^{2+} and Cu^{2+}) or H_2 mode (Fe^{2+}). Each juice was measured in triplicates.

Identification and Quantitation of Polyphenols by HPLC. RPC-MS analysis was performed on an Agilent 1100 HPLC system equipped with a vacuum degasser, a quaternary pump, an autosampler and an UV–Vis diode array detector (all from Agilent Technologies, Santa Clara, USA). Separations were carried out using an ODS Hypersil column (250 mm × 4.6 mm inner diameter; 1.8 µm particle size; Thermo Fisher Scientific, Austria). Analytes were separated by gradient elution with 0.1% (v/v) formic acid (A) and acetonitrile containing 0.1% (v/v) formic acid (B) at a flow-rate of 1 mL/min. The linear gradient elution program was: starting conditions 97.5% A and 2.5% B. The proportion of B was increased to 10% at 20 min, 20% at 32 min, 50% at 45 min and 80% at 50 min. The column was thermostated at 40 °C and the injection volume was 20 µL.

MS measurements were done on a 6520 quadruple/time-of-flight (Q-TOF) instrument equipped with an electrospray ionization source (Agilent Technologies, Santa Clara, USA). Results were obtained using the following settings: MS capillary voltage 3750 V, fragmented voltage 180 V, drying-gas (nitrogen) flow rate 12 L/min, drying-gas temperature 350 °C, and nebulizer pressure 60 psi. Scanning mass range was from *m/z* 70 to 3200 with an acquisition rate of 1.0 spectra/s in the negative MS mode.

Quantitation of identified polyphenols was done using UV absorption by reference substances of known concentrations prepared in deionized water. For reversed phase chromatography (RPC) analysis a Jasco LC-2000 Plus Series system comprising of a quaternary pump with build-in degasser, an autosampler, a temperature controlled column compartment, and a diode array detector (DAD) equipped with Chrompass software (all from Jasco Corporation, Tokyo, Japan) was used. Separation was performed on a Hypersil ODS C18 column (250 mm x 4.6 mm inner diameter, 5 µm particle size; Thermo Fisher Scientific, Vienna, Austria). Column temperature was set to 40 °C and elution was carried out at 1 mL/min. The injection volume for all samples was 20 µL and eluted substances were detected using multiple UV wavelengths from 200 to 350 nm. The following conditions were used for RPC analysis: Mobile phase A contained 0.1% TFA in water. Mobile phase B contained acetonitrile, water and TFA in the ratio 50:50:0.1 (%). Mobile phase C contained 0.1% TFA in acetonitrile. The starting conditions were 95% A and 5% B. Elution was performed with a linear gradient: The proportion of B was increased to 20% at 20 min, 40% at 32 min and 100% at 45 min. Limit of detection (LOD) was defined as signal to noise ratio of 2:1 and limit of quantitation (LOQ) as 4:1. For flavan-3-ols LOD of 0.1 mg/L and LOQ of 0.4 mg/L were defined with a linear range of 1-500 mg/L. For hydroxycinnamic acids LOD of 0.05 mg/L and LOQ of 0.2 mg/L were defined with a linear range of 1-1,000 mg/L. For quercetin derivates LOD of 0.1 mg/L and LOQ of 0.3 mg/L were defined with a linear range of 0.1-100 mg/L. Apple juice samples were centrifuged for 10 min at 15,000 rpm followed by 0.1 µm filtration to remove any remaining solids before analysis.

Quantitation of Acid Content by HPLC. Malic and citric acid content was quantitated for each sample using a RPC method described previously (Shyla, 2011). Quantitation was done using purified malic and citric acid dissolved in deionized water. For RPC analysis a Jasco LC-2000 Plus Series system was used as described above. Separation was performed on a Sorbax SB-C18 column (75 mm x 4.6 mm inner diameter, 3.5 µm particle size; Agilent Technologies, Santa Clara, USA). Column temperature was set to 35 °C and isocratic elution was carried out at 0.5 mL/min. A 50 mM potassium phosphate buffer adjusted to pH 2.8 was used as mobile phase. Apple juice samples were centrifuged for 10 min at 15,000 rpm followed by 0.1 µm filtration to remove any remaining solids before analysis. The injection volume for all samples was 2 µL and eluted substances were detected at 215 nm. Limit of detection (LOD) was defined as signal to noise ratio of

2:1 and limit of quantification (LOQ) as 4:1. For citric acid a LOD of 20 mg/L and LOQ of 50 mg/L were defined with a linear range of 20-1,000 mg/L. For malic acid the LOD was defined at 50 mg/L and the LOQ at 100 mg/L with a linear range of 0.1-20 g/L.

Quantitation of Fruit Acids by Titration. Total acid content was determined using the acidic titration method described in an OECD guideline for food production, which can be found on the OECD website (www.oecd.org/agriculture). In short, apple juice was diluted 1:10 with deionized water, mixed with 3 µL phenolphthalein and titrated with 0.1 M sodium hydroxide until the point of neutrality was reached (indicator changes from colorless to pink). Results were expressed as g/L of malic acid.

Quantitation of Anthocyanin Pigment Content. The anthocyanin content of apple juices was determined using a pH differential method (Weikle, 2012). In brief, apple juices were diluted 1:5 either in pH = 1.0 buffer (0.025 M potassium chloride) or in pH = 4.5 buffer (0.4 M sodium acetate) adjusted with HCl and transferred to 10 mm cuvettes. Absorbance (A) of each diluted sample was determined at 520 and 700 nm within 15 min after preparation for both pH-values. The anthocyanin pigment concentration was calculated as cyanidin-3-glucoside equivalent. The quantitation limit for this method was determined to be 0.1 mg/L. Each juice was measured in triplicates.

Sugar Content Measurements. The sugar content of analyzed apple juice samples was measured using a RHB-55 refractometer (PCE-group, Meschede, Germany). In short, a single drop of undiluted apple juice was loaded onto the prism and the Brix° value was read from the graduation.

Study 2: Characterization and Comparison of Organic Apple Varieties Grown in Lower Austria

Materials and Reagents. (+)-catechin, α-amylase from porcine pancreas, AAPH (2,2´-azobis(amidinopropane) dihydrochloride), ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), acetonitrile, AG1478, bovine catalase, epidermal growth factor (EGF), fluorescein sodium salt, Folin-Ciocalteu reagent, in-vitro toxicology assay kit, methanosulfonic acid, phenolphthalein, potassium persulfate, potassium phosphate, nitric acid, rosmarinic acid, sodium bicarbonate, sodium hydroxide, trifluoroacetic acid (TFA) and Trolox [(±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] were purchased from Sigma Aldrich (Taufkirchen, Germany). Standards for Li^+, K^+, Mg^{2+} NH_4^+ and Ca^{2+} (Certipur, cation multi-element standard III), Cl^-, NO_3^- and SO_4^{2-} (Certipur, anion multi-element standard II) and antimony tartrate solution were from Merck-Millipore (Vienna, Austria). Potassium dihydrogen orthophosphate, ascorbic acid, methanol and ammonium molybdate were purchased from VWR (Vienna, Austria). Sulfuric acid was obtained from Fisher Scientific (Vienna, Austria). Chlorogenic acid, epicatechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate, procyanidin B1, procyanidin B2 and phloridzin were purchased from Extrasynthese (Genay, France). 96-well plates were from Greiner Bio-One (Kremsmünster, Austria). A protein phosphorylation analysis kit termed “cell-based Delfia assay” including an Europium-labeled anti-phosphotyrosine-antibody was obtained from Perkin Elmer (Rodgau, Germany). The Syto62 cell stain reagent was from Life Technologies (Darmstadt, Germany).

Apple Variety Selection and Preparation. 76 apple varieties under study. Out of the 76 apple varieties, 10 varieties were selected for further analysis. The apples were washed and juiced with a juice maker or juiced by hand. The samples were stored at <-60 °C without addition of ascorbic acid. Apples were organically cultivated in an organic orchard located in the Tulin region of Lower Austria. This region is next to the Danue River and is characterized by, on average, 15 days of precipitation each month with May-September having the highest amount of rainfall (mm). This area displays a moderate climate**. Table 2** shows the apples used for this part of the study.

Table 2: 76 apple varieties under study including 10 apple varieties selected for further analysis.

1	Ananas Renette	23	Graue Renette	45	Olderling	67	Seidenbrünnerling
2	Apfel aus Croncels	24	Hausapfel	46	Ontario	68	Selena
3	Bayrischer Brünnerling	25	Hausmütterchen	47	Pilot	69	Siebenkant
4	Berlepsch	26	Ilzer Rosenapfel	48	Plankenapfel	70	Spät blühender Taffelapfel
5	Bohnapfel	27	Ingol	49	Reanda	71	St. Pauler Weinapfel
6	Boikenapfel	28	Jakob Lebel	50	Rebella	72	Taubenapfel
7	Bramleys Seeding	29	Jonathan	51	Remo	73	Taubenapfel Gurten
8	Bratarsch I	30	Kanada Renette	52	Retina	74	Weißer Grießapfel
9	Brauner Matapfel	31	Kanada Renette	53	Rewena	75	Wiltshire
10	Breitarsch II	32	Konstanzer	54	Rewena	76	Zigeuner Apfel
11	Brünnerling	33	Kronprinz Rudolf	55	Rheinischer Krummstiel	**Varieties Selected for Individual Analysis**	
12	Deans Küchenapfel	34	Lansberger Renette	56	Rohling	12	Deans Küchenapfel
13	Edelrambour v. Winnitza	35	Lavanttaler Bananenapfel	57	Roter Boskoop	30	Kanada Renette
14	Enterprize	36	Lederrenette	58	Roter Herbstkalvill	37	Liberty

15	Florianer Rosmarin	37	Liberty	59	Roter Krickapfel	39	Maschanzker
16	Flovina	38	Luna	60	Roter Passamaner	41	Maschschankzka
17	Geheimrat Dr. Oldenburg	39	Maschanzker	61	Roter Settiner	44	Oldenwälder
18	Gewürzluiken	40	Maschanzker	62	Roter Winterrambour	53	Rewena
19	Gloria Mundi	41	Maschschankzka	63	Rubiner	55	Rheinischer Krummstiel
20	Goldparmäne	42	Maunzenapfel	64	Schmidberger Renette	57	Roter Boskoop
21	Goldrenette von Blenheim	43	Minister v. Hammerstein	65	Schöner von Boskoop	64	Schmidberger Renette
22	Goldrush	44	Oldenwälder	66	Schweizer Glockenapfel		

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Technologies, Santa Clara, USA). Separations were carried out using an ODS Hypersil column (250 mm × 4.6 mm inner diameter; 1.8 µm particle size; Thermo Fisher Scientific, Austria). Analytes were separated by gradient elution with 0.1% (v/v) formic acid (A) and acetonitrile containing 0.1% (v/v) formic acid (B) at a flow-rate of 1 mL/min. The linear gradient elution program was: starting conditions 97.5% A and 2.5% B. The proportion of B was increased to 10% at 20 min, 20% at 32 min, 50% at 45 min and 80% at 50 min. The column was thermostated at 40 °C and the injection volume was 20 µL.

MS measurements were done on a 6520 quadrupole/time-of-flight (Q-TOF) instrument equipped with an electrospray ionization source (Agilent Technologies, Santa Clara, USA). Results were obtained using the following settings: MS capillary voltage 3750 V, fragmentor voltage 180 V, drying-gas (nitrogen) flow rate 12 L/min, drying-gas temperature 350 °C, and nebulizer pressure 60 psi. Scanning mass range was from m/z 70 to 3200 with an acquisition rate of 1.0 spectra/s in the negative MS mode.

Identification and Qquantitation of identified polyphenols was done using UV absorption by reference substances of known concentrations prepared in deionized water. For reversed phase chromatography (RPC) analysis a Jasco LC-2000 Plus Series system comprising of a quaternary pump with build-in degasser, an autosampler, a temperature controlled column compartment, and a diode array detector (DAD) equipped with Chrompass software (all from Jasco Corporation, Tokyo, Japan) was used. Separation was performed on a Hypersil ODS C18 column (250 mm x 4.6 mm inner diameter, 5 µm particle size; Thermo Fisher Scientific, Vienna, Austria). Column temperature was set to 40 °C and elution was carried out at 1 0.8 mL/min. The injection volume for all samples was 20 µL and eluted substances were detected using multiple UV wavelengths from 200 to 350 nm. The following conditions were used for RPC analysis: Mobile phase A contained 0.1% TFA in water. Mobile phase B contained acetonitrile, water and TFA in the ratio 5080: 250:0.1 (%). Mobile phase C contained 0.1% TFA in acetonitrile. The starting conditions were 9597% A and 53% B. Elution was performed with a linear gradient: The proportion of B was increased to 20% at 20 min, 40% at 32 min and 100% at 45 min. Limit of detection (LOD) was defined as signal to noise ratio of 2:1 and limit of quantitation (LOQ) as 4:1. For flavan-3-ols LOD of 0.1 mg/L and LOQ of 0.4 mg/L were defined with a linear range of 1-500 mg/L. For hydroxycinnamic acids LOD of 0.05 mg/L and LOQ of 0.2 mg/L were defined with a linear range of 1-1,000 mg/L. For quercetin derivates LOD of 0.1 mg/L and LOQ of 0.3 mg/L were defined with a linear range of 0.1-100 mg/L. Apple juice samples were centrifuged for 10 min at 15,000 rpm followed by 0.1 µm filtration to remove any remaining solids before analysis.

Firmness.

Ripeness of the apples was determined using an analogue fruit pressure tester FT327 (Faccini, Italy) according to the manufactures instructions. In short, on diametric points small parts of the peel were removed using the enclosed fruit peeler. Using the 1 cm square tip the pressure was measured and reported in kg.

Quantitation of Fruit Acids by Titration. Total acid content was determined using the acidic titration method described in an OECD guideline for food production, which can be found on the OECD website (www.oecd.org/agriculture). In short, apple juice was diluted 1:10 with deionized water, mixed with 3 µL phenolphthalein and titrated with 0.1 M sodium hydroxide until the point of neutrality was reached (indicator changes from colourless to pink). Results were expressed as g/L of malic acid.

Quantitation of Acid Content by HPLC. Malic and citric acid content was quantitated for each sample using a RPC method described previously (Weikle, 2012). Quantitation was done using purified malic and citric acid dissolved in deionized water. For RPC analysis a Jasco LC-2000 Plus Series system comprising of an analytical pump with external degasser, autosampler, temperature controlled column compartment and UV-Vis detector equipped with Chrompass software (all from Jasco Corporation, Tokyo, Japan) was used. Separation was performed on a Sorbax SB-C18 column (75 mm x 4.6 mm inner diameter, 3.5 µm particle size; Agilent Technologies, Santa Clara, USA). Column temperature was set to 35 °C and isocratic elution was carried out at 0.5 mL/min. A 50 mM potassium phosphate buffer adjusted to pH 2.8 using concentrated phosphoric acid was used as mobile phase. Apple juice samples were centrifuged for 10 min at 15,000 rpm followed by 0.1 µm filtration to remove any remaining solids before analysis. The injection volume for all samples was 2 µL and eluted substances were detected at 215 nm. Limit of detection (LOD) was defined as signal to noise ratio of 2:1 and limit of quantification (LOQ) as 4:1. For citric acid a LOD of 20 mg/L and LOQ of 50 mg/L were defined with a linear range of 20-1,000 mg/L. For malic acid the LOD was defined at 50 mg/L and the LOQ at 100 mg/L with a linear range of 0.1-20 g/L.

Quantitation of Anthocyanin Pigment Content. The anthocyanin content of apple juices was determined using a pH differential method.18 In brief, apple juices were diluted 1:5 either in pH = 1.0 buffer (0.025 M potassium chloride) or in pH = 4.5 buffer (0.4 M sodium acetate) adjusted with HCl and transferred to 10 mm cuvettes. Absorbance (A) of each diluted sample was determined at 520 and 700 nm within 15 min after preparation for both pH-values. The anthocyanin pigment concentration was calculated as cyanidin-3-glucoside equivalent. The quantitation limit for this method was determined to be 0.1 mg/L. Each juice was measured in triplicates.

Sugar Content Measurements. The sugar content of analysed apple juice samples was measured using a RHB-55 refractometer (PCE-group, Meschede, Germany). In short, a single drop of undiluted apple juice was loaded onto the prism and the Brix° value was read from the graduation.

Ferric Reducing Ability of Plasma (FRAP) measurements. The FRAP assay was performed based as described by Benzie and Strain. 23 FRAP reagent was prepared by mixing 10 parts acetate buffer (300 mM, pH 3.6) with 1 part 2-4-6-tripyridyl-s-triazine (10 mM in 40 mM HCl) and 1 part iron(III)chloride-hexahydrate (20 mM in ddH2Odeionized water) and used immediately. Apple juices were diluted 1:10 in ddH2Odeionized water. 300 µL of FRAP reagent were mixed thoroughly with 10 µL diluted samples. Absorbance was measured at 593 nm immediately and after 10 min incubation at 37 °C using a plate reader device. FRAP results for each sample were calculated using a dilution series of Trolox. As a positive control for each assay a 1 mM ascorbic acid standard was analyzedincluded. Each juice was measured in triplicates

Results and dicussion: Study 1 and Study 2

Study 1- Bioanalytical Characterization of 88 apple varieties from Upper Austria

Physicochemical Properties of Investigated Apple Varieties.

Study one harvested apples in Upper Austria from an organic orchard. Apples were analysed and for various properties and investigated the comparison of apples which were grafted as well as investigated the EGFR activity.

The ripeness of the apple juices was investigated. Apple variety ripeness was compared by quantitation of the sugar content (Brix° value) and the sample acidity (titratable acidity (TA), malic and citric acid). **Table 3** displays a mean Brix° value of 12.9 ranging from 8.0 to 18.9, and a mean titratable acidity of 0.8 (% as malic acid) ranging from 0.27 to 1.95 was found. These results indicate a high sugar-acid-ratio. This is in agreement with a ripe state as specified by the FAO. The values for the analyzed apple juices can be found in **Supplementary Table 1**. All samples are characterized by high sugar levels, and the majority of samples have been found to have low acidity contents. Some apple juice varieties, e.g. Zuccalmaglios Renette or Geheimrat Oldenburg, demonstrated to have comparatively high, variety-specific acidity levels. Nevertheless, those samples displayed Brix° values within an acceptable range. Overall, complied data indicates that all apples were mature at time of harvest.

Table 3: Ripeness parameters (Brix°, titratable acidity, malic acid, citric acid) of apple juice prepared from 88 apple varieties.

	Units	Mean	SD	%CV	Minimum	Maximum	Range
Brix°		12.9	2.0	15.2	8.0	18.9	10.9
TA	(% as malic)	0.80	0.31	36.90	0.27	1.95	1.68
Malic acid	(mg/L)	7175.4	4261.4	59.4	127.5	17245.1	17117.6
Citric acid	(mg/L)	115.9	91.6	79.0	28.4	522.6	494.2

As seen in **Table 4**, a significant variability for TPC was found ranging from 103.2 mg/L up to 2.3 g/L in some varieties. Refer to **Supplementary Table 1** for the complete value set. The mean TPC of all 88 varieties was determined to be 777.7. A large standard deviation of 447.2 mg/L emphasizes the significant differences of the polyphenol content among the apple varieties. Results show that the TPC levels of certain, popular apple varieties are comparatively low. For example, the TPC content of juice prepared from Royal Gala and Topaz apples grown in the Eferding-region was found to be only 185.8 and 257.4 mg/L, respectively. Gala apples are among the most commonly sold apple varieties worldwide, and are thenumber two sellers on the United States market. Likewise, Topaz apples are highly popular, especially in Central Europe. It's unfortunate that these commonplace apples do not display high TPC values when compared to other cultivars. This means that the average purchase, and therefore, consumption, of apples potentially provides only a fraction of the health benefits that other cultivars can provide.

Table 4: Ripeness parameters (Brix°, titratable acidity, malic acid, citric acid) of apple juice prepared from 88 apple varieties.

	Units	Mean	SD	%CV	Minimum	Maximum	Range
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In contrast, the majority of the apple varieties (~60%) that were investigated within this study are used as dessert apples, 25% for the production of non-alcoholic or alcoholic beverages, and 15% as industrial apples that can be e.g. utilized for food production. However, the economic significance of most of these apple varieties is rather low and limited to the region of Eferding in Upper Austria. Thus, broader distribution of selected dessert apples varieties with high TPC levels as characterized in this study (e.g. Harberts Renette, Odenwälder and Zuccalmaglios Renette) should be attempted.

However, when investigating TPC values, it is important to note the drawbacks of using the FC-method. A drawback of the FC-method is that other reducing reagents present in the apple juice supernatant, such as ascorbic acid, might lead to an overestimation of obtained TPC values (Lee, 2005). In addition, being a major feature of this study, the assay is not sufficient to predict the antioxidant effect of apple juice, since biological effects are to be expected from different polyphenols. To address this question, the exact composition of the polyphenol components has to be unraveled. Importantly, using the Phenol-Explorer, information on the polyphenol content could only be extracted for two apple varieties that are included in this study, namely *Royal Gala* and *Grüner Boskoop* (McCue, 2004). However, a direct comparison to our results appears difficult due to application of juice extracts for these studies rather than analyzing untreated apple juice (Hollman 2011, Rothwell 2013).

Table 5: Single polyphenol content (mg/L) of apple juices under study.

	Mean	**SD**	**%CV**	**Minimum**	**Maximum**	**Range**
Chlorogenic acid	216.3	223.8	103.5	+	1209.2	1209.2
Caffeic acid	3.8	4.4	116.4	+	32.5	32.5
4-*p*-Coumaroylquinic acid	11.7	13.3	114.7	+	55.5	55.5
Σ Hydroxycinnamic acids	**231.8**					
Phloretin-2'-*O*-xyloglucoside	12.8	10.1	78.8	+	54.3	54.3
Phloridzin	6.8	5.9	85.6	+	29.5	29.5
Σ Dihydrochalcone derivates	**19.6**					
Procyanidin B1	3.5	5.2	147.6	+	23.0	23.0
Procyanidin B2	40.1	48.3	120.4	+	338.1	338.1
(-)-Epicatechin	13.3	17.5	131.8	+	104.7	104.7
Epigallocatechin	3.3	6.6	200.8	+	38.2	38.2
Epichatechingallate	11.3	10.4	91.9	+	51.0	51.0
Σ Flavan-3-ols	**71.5**					

Quercetin-3-*O*-galactoside	0.7	2.9	396.7	+	26.4	26.4
Quercetin-3-*O*-xyloside	2.5	4.9	194.8	+	41.1	41.1
Quercetin-3-*O*-rhamnoside	2.4	3.3	137.2	+	25.2	25.2
Quercetin	3.0	5.9	196.0	+	49.2	49.2
Quercetin-3-*O*-rutinoside	0.3	1.8	580.6	+	15.7	15.7
Σ Flavonols	**8.9**					
Σ Anthocyanins	**1.01**	1.41	139.41	0.13	6.79	6.66
Total polyphenol amount (HPLC)	**331.8**					

n.d., not detectable; +, < limit of quantitation

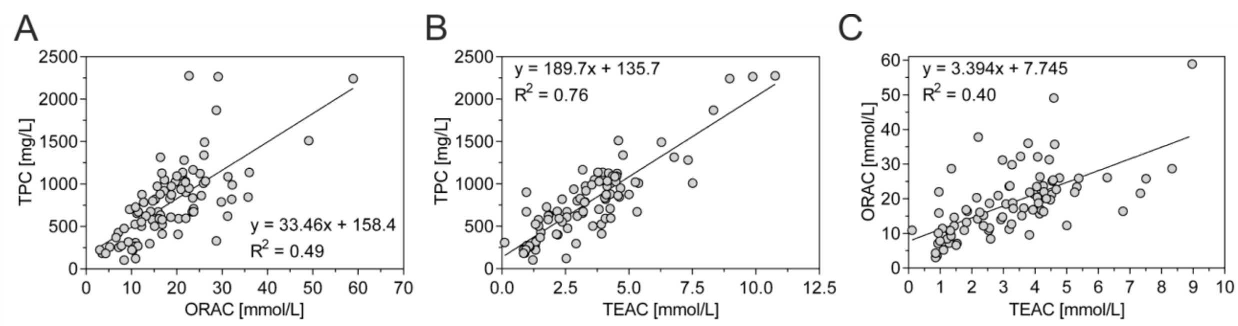
Fifteen polyphenolic compounds belonging to four different major polyphenol groups were identified in the apple juices prepared from each variety: Chlorogenic, caffeic- and 4-*p*-coumaroylquinic acid (hydroxycinnamic acids), phloretin-2'-*O*-xyloglucoside and phloridzin (dihydrochalcone derivates), procyanidin B1 and B2, (-)-epicatechin, epigallocatechin and epicatechingallate (flavan-3-ols), and five quercetin derivates (flavonols). A representative HPLC-DAD diagram, indicating retention times and maximal wavelengths of each compound, is shown in **Supplementary Figure 1**. **Table 5** summarizes the content of selected polyphenolics of all 88 apple juice samples. In addition, obtained values for each variety can be found in **Supplementary Table 2**.

Among the five investigated polyphenolic groups, the hydroxycinnamic acid group was found to be the most abundant one, with chlorogenic acid being the main compound in this polyphenol group. The ratio between chlorogenic acid and p-coumaroylquinic acid content in different varieties has been reported to vary between 37.1 and 1.2 (Podsedek, 2000).Those ratio limits are in good agreement with the mean ratio of 20.3 of the apple juice samples presented in this study. However, for some samples much higher ratios up to 104.1 (L203) or 181.5 (L214) were found, which was mainly caused by high levels of chlorogenic acid and at the same time a very low concentration of p-coumaroylquinic acid being detected in these varieties. The low amounts of caffeic acid found in most samples are in good agreement with other studies (Vrhovsek, 2004)

Flavan-3-ols represent the second largest group of polyphenols detected in the investigated apple juices. Procyanidin B2 and epicatechin were the most abundant polyphenolic substances in this group with a mean concentration of 40.1 and 13.3 mg/L, respectively. Similar amounts were reported in a previous study analyzing untreated apple juices (Kahle, 2005). Two dihydrochalcone derivates were found in most apple juices: With a mean content of 6.8 mg/L phloridzin and 12.8 mg/L phloretin-2'-*O*-xyloglucoside, our results are within the range of a previous study (~1-25 mg/L each). Our measurements also confirmed the presence of flavonols. However, this polyphenol group was found in very low concentrations (~2% of total polyphenol amount). Anthocyanin pigments were found only in a few juice samples at low concentrations ranging from 0.13 to 6.79 mg/L. This is in line with the presence of these phytochemicals exclusively in apples characterized by a red skin (Eisele, 2005).

In agreement with the results from TPC measurements, RPC analysis unraveled great differences in the polyphenol content and composition of apple juices from different apple varieties. For example, the amount of chlorogenic acid for some varieties ranged from 1.79 mg/L (Royal Gala, F204) up to 1209.7 mg/L (Harberts Renette). In general, the estimated TPC values correlate with the concentration of polyphenols found by HPLC measurements. However, a high TPC level does not necessarily correlate with the detected amounts of the analyzed single polyphenols. For example, the varieties F213, L202 and L203 are all characterized by a similar TPC value of about 1.000 mg/L. However, the concentration of chlorogenic acid was found to vary between 76.4, 149.73 and 145.89 mg/L, respectively. Thus, our results clearly showed that juices prepared from various apple varieties are highly diverse in their content of total polyphenols and show great variations in their individual polyphenol composition. Furthermore, when comparing different apple varieties grafted on the same tree (F- and L series, respectively), it could be observed that these varieties retain their individual polyphenolic profile. The described differences are likely to depend mainly on genetic factors, which are consistent with a study analyzing the genetic variability of apples (Gliszczynska-Swiglo, 2003). In conclusion, grafting proves to be a superior tool for a fast growth of selected apple varieties with varying phytochemical concentration, without the need for the time-consuming cultivation of the whole tree.

Figure 1



Two different methods (TEAC and ORAC) were used to measure the total antioxidant capacity of the different apple juices. **Table 6** summarizes the TEAC and ORAC values from all samples. The antioxidant capacity of these juices ranged from 0.8 to 7.8 mM (TEAC) and 3.0 to 58.9 mM (ORAC), which is in agreement to similar studies (Volz, 2011). The full list of TEAC and ORAC values from all apple juice samples can be found in **Supplementary Table 1**. Results indicate the general dependence of the antioxidant capacity on the total polyphenolic concentration as can be seen by the linear regression and correlation analysis. The coefficients of determination (R^2) are given in **Figure 1**. All R-values (correlation coefficients) were positive at the $P < 0.0001$

significance level, indicating that the values of antioxidant capacities, assayed by the two different methods, were highly correlated (R-values for TPC/ORAC: 0.69; TPC/TEAC: 0.87; ORAC/TEAC: 0.63). The regression coefficient value obtained for TPC and ORAC assay was lower compared with TEAC assay, but significant in both systems. These results indicated that the two assays were suitable and reliable for assessing total antioxidant capacities. Thus, despite the aforementioned limitations of the FC-method to determine the TPC, it can possibly be considered as a first indicator of antioxidant capacity of apple juice.

Table 6: Total phenolic content (TPC) and antioxidant capacities of apple juices under study.

	Units	Mean	SD	%CV	Minimum	Maximum	Range
TPC	(mg/L)	777.7	447.2	57.5	103.2	2275.6	2172.4
TEAC	(mmol/L)	3.3	1.6	48.3	0.8	7.8	6.9
ORAC	(mmol/L)	18.8	9.5	50.5	3.0	58.9	55.9

It was observed that some varieties contained equal (TPC of Lesans Kalvill similar to Samareiner Rosmarien) or even more total polyphenols than others (TPC Riesenboikenapfel > Glasapfel), but lower antioxidant capacity as determined by both, TEAC and ORAC measurements. This effect can be explained by the different antioxidant activity of individual polyphenols as reported in previous studies (Tsao, 2005).

Consequently, the antioxidant capacity is dependent on the phenolic composition of an individual juice.

The low TEAC and ORAC values of juice prepared from certain apple varieties, including Topaz, is consistent with other studies analyzing the antioxidant capacity of a number of old and new apple varieties in Poland (Karaman, 2013). The Topaz apple is a good example of a new apple variety (introduced in the 1980's), which offers several advantages for agriculture including resistance to apple scab, high yields and good storage properties. It is also a common apple variety for organic farming. However, the low level of phenolic substances in these varieties has not been taken into consideration so far. Consequently, the distribution of apple varieties with a higher content of polyphenols should be supported to promote their positive effects on human health.

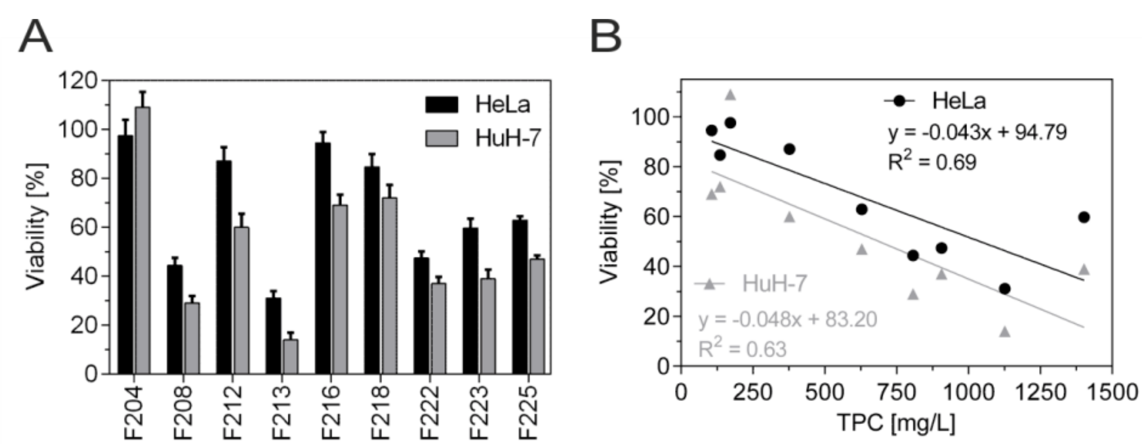
The mean and range values of the major minerals K^+, Mg^{2+} and Ca^{2+} are indicated in **Table 7**, results for individual varieties can be found in **Supplementary Table 1**. The potassium concentration ranged from 620.4 (Retina) to 2064.0 mg/L (Grüner Boskoop) with large variations between individual varieties. Compared to potassium, the concentration of magnesium and calcium was significantly lower ranging between 8.4 and 64.4 (Mg^{2+}) and 6.7 to 57.5 (Ca^{2+}) mg/L, respectively. Similar to the potassium concentration, remarkable variations for calcium and magnesium between the different varieties could be observed. Collected data, especially for potassium and copper, are in good agreement with other studies analyzing minerals and trace elements of different apple varieties (Vrhovsek 2004, Sun 2002).

Table 7: Mineral content of apple juices under study.

	Units	Mean	SD	%CV	Minimum	Maximum	Range
K^+	(mg/L)	1082.7	23.5	23.5	620.4	2064.0	1443.6
Mg^{2+}	(mg/L)	30.7	1.2	45.0	8.4	64.4	56.1
Ca^{2+}	(mg/L)	23.7	0.8	41.8	6.7	57.5	50.9
Cu^{2+}	(µg/L)	320.3	9.7	35.9	109.8	572.2	462.5
Mn^{2+}	(µg/L)	307.9	18.4	70.8	59.5	688.5	629.0
Fe^{2+}	(µg/L)	268.3	10.5	46.5	130.0	670.0	540.0
PO_4^{3-}	(mg/L)	209.7	5.3	33.9	90.0	420.3	330.3

Fifty-two apple juice samples were analyzed for their copper and manganese content and 27 (F- and L series) for iron, respectively. The observed variations between individual samples, especially those for Mn^{2+} and Fe^{2+}, were highly pronounced for these elements. The mean and range values for the Cu^{2+}, Mn^{2+} and Fe^{2+} content are summarized in **Table 7**. Taken together, our results show large variations between the different apple varieties, which is in good agreement with a previous study analyzing the concentration of various minerals in juice prepared from 175 apple varieties (Vrhovsek, 2004). These variations can also be observed for various apple varieties grafted on a single tree (F- and L-series). Thus, apples harvested from grafted trees not only retain their polyphenolic profiles, but also their characteristic mineral-, trace element- and phosphate-concentrations. From this point of view an intensified cultivation of selected apple varieties identified in this work should be considered. However, the availability of many of the varieties that possess these positive compositions for large scale cultivation remains a limiting factor. Engrafting turned out to be an excellent way to enhance growth rates and provide resistance to bacterial or fungal infections. By engrafting 27 different apple varieties on two trees grown close to each other, it could be shown that the apple fruits in fact remain their primary ingredient characteristics. This fact is clearly of key importance for the promotion of selected apple varieties.

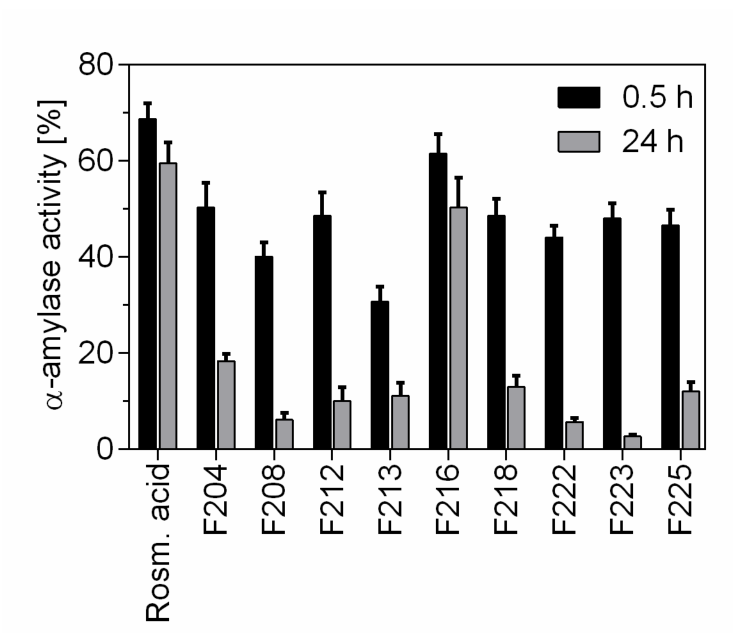
Figure 2



Different assays were used to determine several biological effects of apple juice varieties that were preselected by a pronounced variation of different ingredients including polyphenols, minerals and trace elements. Juices from nine apple varieties grafted on a single tree (F-series) were used for these analyses. First, the cytotoxic effects of apple juice were determined on two different human cancer cell lines using a resazurin based assay. Apple juice has been reported to be a strong cancer chemo-preventive (Wojdylo, 2008). Several studies have already shown the inhibitory effect on cell proliferation of cultured cancer cell lines (Abid, 2014). Two human cell lines were used for investigation of the growth inhibition of selected apple juices. As shown in **Figure 2A** a strong reduction of cell viability could be observed in both cell lines for several apple juices at a 1:5 dilution. The observed inhibitory effect was clearly dependent on the apple juice concentration: no reduction in cell viability could be observed at higher dilution rates >1:50 (**Supplementary Figure 1**). Using apple juice in cell culture medium might lead to H_2O_2 formation and pronounced cytotoxic effects (Gerhauser, 2008). Addition of catalase (100 U/mL) to prevent the formation of H_2O_2 further slightly reduced these cytotoxic effects (data not shown). Interestingly, a clear correlation between TPC levels and the described cytotoxic effects was found (**Figure 2B**). Our results show that certain apple juices reduce the viability of the analyzed human cancer cell lines in a TPC dependent manner. Interestingly, Veeriah et al. showed that native apple extracts were about twice as potent as a composed mixture of low molecular weight apple polyphenols in inhibiting cancer cell growth (Abid, 2014). This indicates that other constituents, such as oligomeric procyanidines, substantially contribute to the potent anti-proliferative properties of polyphenol-rich apple juices. Thus, the usage of apple juices instead of extract appears straightforward.

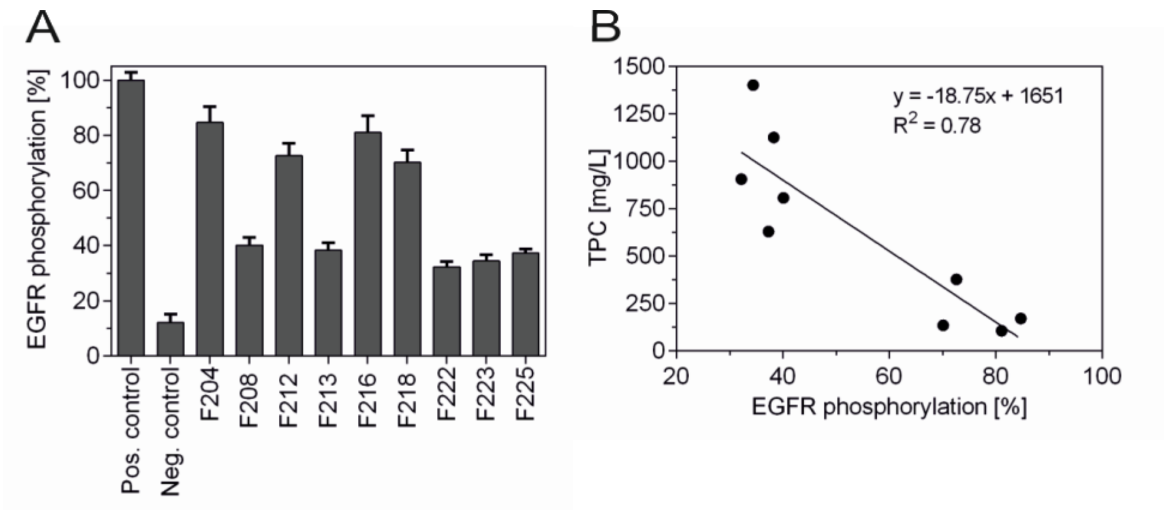
In a second experiment a putative apple juice dependent inhibition of human α-amylase, a major digestive enzyme that breaks down long-chain carbohydrates, was evaluated. Salivary and pancreatic α-amylases lead to the formation of maltose and other related oligomers by catalyzing the hydrolysis of α-1,4-linked glucose chains (Veeriah, 2006). Several studies indicated a beneficial health effect of bioactive substances from apples, e.g. a reduced risk of chronic diseases including type 2 diabetes. In this regard the inhibition of α-amylase activity by these substances is of particular importance (Lapidot, 2002). As shown in **Figure 3** a 30-40% inhibition of α-amylase-activity was observed when rosmarinic acid (1.5 mg/mL) was added, confirming the inhibitory potential of this substance. All tested apple juices of the F-series also showed an incubation-time dependent inhibition of α-amylase-activity between ~80% (24 hours incubation) and ~50% (30 min incubation). In these experiments differences between individual varieties ranging from 40-70% (30 min) and 50-98% (24 hours) inhibition of α-amylase-activity could be observed. In contrast to the antioxidant capacity, the observed inhibitory effect was not dependent on the TPC levels of the apple juice varieties as statistical analysis determining Kendall's and Spearman's rank correlation coefficient did not reveal any significant correlation (data not shown). These results are in good agreement with a recent study excluding a positive correlation between α-amylase inhibitory activity and total phenolic content (Lapidot, 2002). However, single polyphenols that have been shown to inhibit this enzyme (e.g. chlorogenic acid) are found at high concentrations in our apple varieties (Hanhineva, 2010).

Figure 3



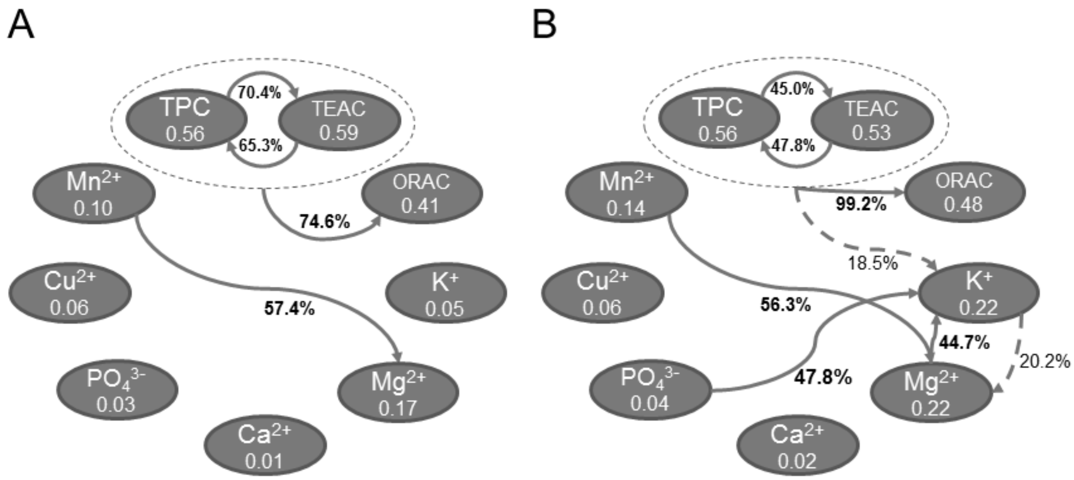
Finally, the effects of different apple juice varieties on the activity of the epidermal growth factor receptor (EGFR) were analyzed. The EGFR plays a major role in cellular signaling: insufficient signaling may lead to the development of neurodegenerative diseases, while excessive EGFR signaling is associated with the development of a wide variety of tumors (Barbosa, 2010). Highly elevated EGFR signaling seems to be a critical factor in the development and malignancy of these tumors (Sales, 2010). Several studies have indicated that apple polyphenols inhibit EGFR activity in various cell lines (Bublil 2007, Cho 2002, Fridrich 2007). Thus, apples rich in polyphenols are thought to prevent the formation of various cancer types in the human body. In total nine apple juice varieties of the F-series were analyzed for a potential phosphorylation-inhibition of the EGFR. As shown in **Figure 4A** the applied time-resolved-fluorescence based ELISA-assay is well-suited to detect phosphorylation of the EGFR upon stimulation. AG1478 pre-treatment for 4 hours clearly inhibited the phosphorylation upon EGF stimulation. When cells were incubated for 4 hours with juice from different apple varieties (1:20; in the presence of catalase), a pronounced inhibition of EGFR phosphorylation could be observed. Our experiments indicated remarkable differences in the degree of phosphorylation inhibition depending on the used apple varieties. Further analysis showed that the observed inhibitory effects significantly correlated with the respective TPC levels (**Figure 4B**), which is in consistence with similar studies. Individual polyphenols such as the procyanidin dimers B1 and B2 or phloretin and phloretin-2'-*O*-xylogucoside have been found to specifically affect the EGFR activity (Bublil 2007, Cho 2002, Fridrich 2007). Accordingly, apple juice varieties that inhibited the EGFR activity to the highest extent (F208, F213 and F223) were especially rich in these polyphenols.

Figure 4



The described dependence of the antioxidant capacity on the TPC levels is in agreement with previous studies (Lee, 2003). This research was extended by the use of mathematical modeling that allowed us to identify variable interaction networks based on the analysis of apple components data. Regression models that approximate selected target variables using other available parameters in this data set have been identified. The relevance of a variable in this context can be defined via the frequency of its occurrence in models identified by evolutionary machine learning methods or via the decrease in modeling quality after removing it from the data set (Kern, 2005). The following algorithms have been applied for the data set generated in this study including the results for TPC, TEAC, ORAC, Mn^{2+}, Mg^{2+}, Ca^{2+}, Cu^{2+}, K^{+} and PO_4^{3-}: Linear regression and random forests (Teller, 2013). As shown in **Figure 5A**, linear regression confirms the relationship of the antioxidant capacity (TEAC and ORAC) and the TPC level. In addition, a significant interrelationship of Mg^{2+} and Mn^{2+} was found. These findings were confirmed when non-linear modeling using random forest was applied (**Figure 5B**). The latter model also indicated a strong relevance of PO_4^{3-} and Mg^{2+} on the modeling of K^+. Even though importance in regression and correlation do not imply causality, this analysis implies that apples that are rich in Mg^{2+} by trend also contain higher levels of Mn^{2+}. The same assumption holds true for K^+ and PO_4^{3-}. Mn^{2+} and Mg^{2+} are abundant elements and essential to all living cells. For example Mg^{2+} plays a major role in manipulating biological compounds including DNA, RNA and ATP. In addition, a great number of enzymes require Mg^{2+} for their function. The same is true for Mn^{2+} ions which are essential cofactors for many enzymes. However, many of these enzymes can use Mg^{2+} as a replacement of Mn^{2+} (Winkler, 2013). Of special interest is the function of Mn^{2+} enzymes to detoxify superoxide free radicals in mitochondria (Breiman, 2001). In analogy copper and zinc bound enzymes are necessary for detoxification in the cytosol (Crowley, 2000). Thus, similar to polyphenols, Mn^{2+} and Cu^{2+} ions play a key role in preventing the human organism from oxidative damage.

Figure 5



A
TPC
0.56
70.4%
65.3%
TEAC
0.59
Mn2+
0.10
ORAC
0.41
74.6%
Cu2+
0.06
57.4%
K+
0.05
PO43-
0.03
Mg2+
0.17
Ca2+
0.01
B
TPC
0.56
45.0%
47.8%
TEAC
0.53
Mn2+
0.14
99.2%
ORAC
0.48
18.5%
Cu2+
0.06
56.3%
K+
0.22
44.7%
20.2%
PO43-
0.04
47.8%
Mg2+
0.22
Ca2+
0.02

Study 2 – Biochemical Composition of 76 apple varieties grown in Lower Austria

Determination of ripeness and sugar content

Study 2 invesigated 76 apple cultivars harvested from an orchard in Lower Austria. Like apple cultivars were compared with Study 1. Cultivars in Study 2 were subjected to random selection and would under go analysis of different apples from like cultivars. Study 2 helped reienforce basic ideas and correlations identified in Study 1.

As mentioned, the maturity level of a fruit has an effect on the phytochemicals present within that fruit. Consequently, the maturity levels of apple juices were tested to ensure that levels of phytochemicals within apple cultivars can accurately be compared. The guidelines presented by the FAO, which help determine level of maturity, were used. All juices presented were determined to be ripe using the starch index. Each juice had negative starch values; therefore, a value of 10 was used for the starch value indicated in Equation 1. After looking at the averaged CV's of the 76 cultivars in question, it can be determined that the apples show low variation in maturity levels. Starch index values displayed a CV% of 37.58. This indicates a medium variation between apple cultivar maturity levels. Optimally, comparison of apple cultivars would show a CV of 0. Significance of this difference has yet to be identified in previous literature and it is recommended to further this study.

Equation 1: Starch Index

$$Starch\ Index = \frac{Firmness}{Brix° \times Starch\ Value}$$

Analysis of the 76 cultivars resulted in a mean Brix° value of 12.79 with a mean titratable acidity (TA) of 0.82 (% as malic acid) as seen in **Table 8**. Average malic acid content was determined to be 4747.55 mg/L with a high of 22421.60 mg/L, seen in the St. Pauler Weinapfel. This is expected because St. Pauler Weinapfel is a wine apple, and for production purposes, wine apples should contain high levels of malic acid. Citric acid content averaged 89.97 mg/L with a high of 987.70 mg/L (Ilzer Rosenapfel) and a low of 5.20 mg/L (Rohling). The coefficient of variation in percentages (%CV) in Brix° (10.08), TA (29.57), malic acid (92.21), citric acid (139.66), firmness (39.36) and strife index (37.58) were identified. Additional values for the analyzed apple juices can be found in **Supplementary Table 3**. High %CV values between cultivars are expected in acid contents because normalized or base line acid levels vary greatly between cultivar. The acid levels also can be indicative of what they can be used for- as exemplified in the wine apples.

Table 8: Ripeness parameters (Brix°, titratable acidity (TA), malic acid, citric acid, firmness) of apple juice prepared from 76 apple varieties.

	Units	Mean	SD	%CV	Minimum	Maximum	Range
Brix°		12.79	1.29	10.08	10.00	15.67	5.67
TA	(% as malic)	0.82	0.24	29.57	0.54	1.74	1.21
Malic acid	(mg/L)	4747.55	4377.53	92.21	68.60	22421.60	22353.00
Citric acid	(mg/L)	89.97	125.65	139.66	5.20	987.70	982.50
Firmness	kg	6.49	2.55	39.36	2.15	13.88	11.73
Strife Index		0.05	0.02	37.58	0.02	0.10	0.08

Analysis of phenolic contents and antioxidant capacity

The 76 varieties also were analyzed for total phenolic content (TPC) and the antioxidant capacities of juices were analyzed. A %CV value of 66.98 within TPC values, as indicated in Table 3, highlights the large variation in TPC content between apple cultivar. This means that some of the cultivars have higher TCP content than others which can potentially indicate the health potential of each cultivar. FRAP and ORAC values were measured to determine the antioxidant capacity of the apple juices. FRAP measurements were taken instead of TEAC measurements, because FRAP is proven to be the superior method of analysis for our target (Zulueta et al, 2009). FRAP measurements had a mean value of 14.80 mmol/L and ORAC measurements had a mean value of 18.10 mmol/L. FRAP values between cultivars showed a lower %CV (58.98) compared to ORAC values (72.96). These values should be re-measured to ensure accuracy; however, the average CV value is reduced significantly when rare outliers are removed.

As mentioned, ORAC and FRAP measurements represent the antioxidant capacities within fruits. Positive and significant correlations between TPC versus ORAC and TPC versus FRAP indicates a dependence of antioxidant capacities on the total phenolic content, seen in **Figure 6** and **Figure 7**. Values can be reviewed in **Table 9.** This is significant because this represents both the ferric reducing ability and oxygen radical absorbance capacity's dependence on the total phenolic content within apple juices. A study also analyzing apple juices determined the dependence of the Trolox-equivalent antioxidant capacity (TEAC) assay on total phenolic content (Gliszczynska-Swiglo, 2003) (Cao, 1998). These correlations indicate protection against dangerous levels of oxidation in the body if apple juice is consumed.

Figure 6: TCP vs ORAC values in 76 apple varieties

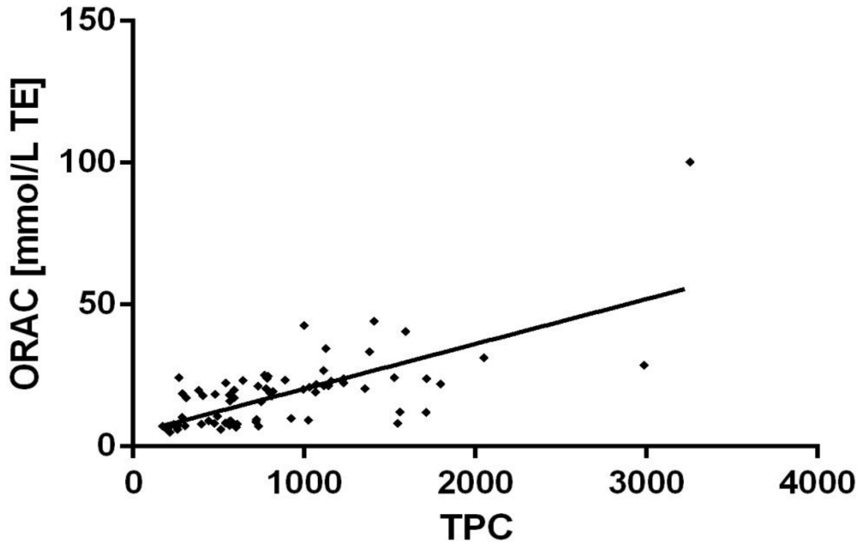


Figure 7: TCP vs FRAP values in 76 apple

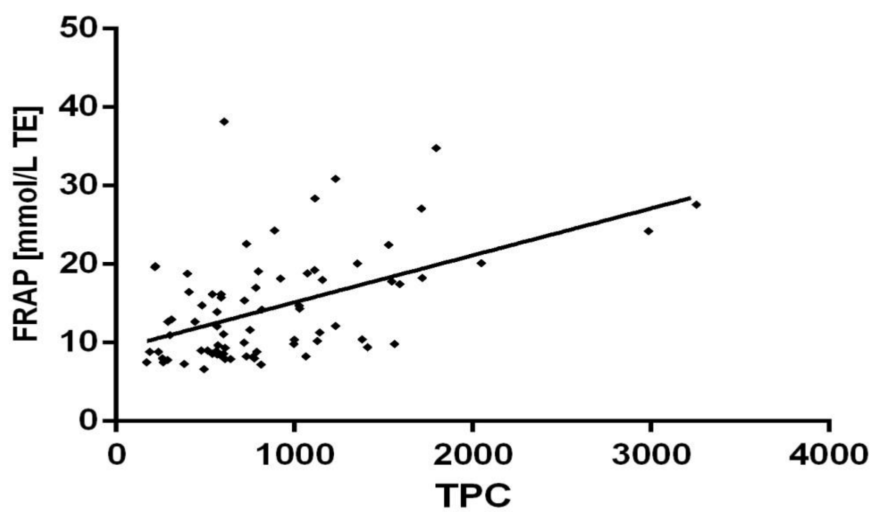


Table 9: Total phenolic content (TPC) and antioxidant capacities of apple juices under study.

	Units	Mean	SD	%CV	Minimum	Maximum	Range
TPC	(mg/L)	857.80	574.54	66.98	173.50	3254.80	3081.3
FRAP	(mmol/L)	14.80	8.71	58.98	6.60	62.4	55.70
ORAC	(mmol/L)	18.10	13.21	72.96	5.00	100.3	95.20

Identification and quantification of single phenolic compounds by HPLC analysis

Single polyphenol content of untreated and water soluble apple juices under investigation resulted in the discovery of 15 compounds in varying polyphenol groups. The polyphenol groups identified include: Hydroxycinnamic acids, Dihydrochalcone derivates, Flavan-3-ols, and Flavonols. Values of single polyphenol content presented in mg/L can be reviewed in **Table 10**. Due to their water soluble characteristics, these values are a representation of bio-available substances present.

Table 10: Single polyphenol content (mg/L) of apple juice under study.

	Mean	SD	%CV	Minimum	Maximum	Range
Chlorogenic acid	118.90	123.98	1.04	0.00	495.24	495.24

Caffeic acid	1.82	1.93	1.06	0.00	9.04	9.04
4-*p*-Coumaroylquinic acid	5.11	8.86	1.73	0.00	60.50	60.50
Σ Hydroxycinnamic acids	**125.84**					
Phloretin-2’-*O*-xyloglucoside	7.27	10.96	1.51	0.00	53.35	53.35
Phloridzin	3.89	4.91	1.26	0.00	24.05	24.05
Σ Dihydrochalcone derivates	**11.16**					
Procyanidin B1	4.68	7.70	1.65	0.00	58.81	58.81
Procyanidin B2	19.39	27.80	1.43	0.00	151.67	151.67
(-)-Epicatechin	10.52	21.13	2.01	0.00	128.00	128.00
Epigallocatechin	2.44	2.97	1.22	0.00	18.92	18.92
Epichatechingallate	6.72	7.12	1.06	0.00	36.41	36.41
Σ Flavan-3-ols	**43.74**					
Quercetin-3-*O*-galactoside	1.70	1.71	1.00	0.00	6.87	6.87

Quercetin-3-*O*-xyloside	1.57	1.53	0.98	0.00	7.51	7.51
Quercetin-3-*O*-rhamnoside	0.48	0.57	1.19	0.00	2.88	2.88
Quercetin	1.27	1.35	1.06	0.00	5.91	5.91
Quercetin-3-*O*-rutinoside	0.22	0.32	1.45	0.00	1.44	1.44
Σ Flavonols	**5.25**					
Σ Anthocyanins	**1.1**	2.15	2.05	n.d.	9.5	9.5
Total polyphenol amount (HPLC)	**187.09**					

n.d., not detectable; +, < limit of quantitation

After analysis, it was determined that hydroxycinnamic acids make up 67% of the total quantified polyphenols. Chlorogenic acid is the main contributor with a mean of 118.90 mg/L. Flavan-3-ols make up 23% of all polyphenols with Procyanidin B2 contributing the highest amount of 19.39 mg/L and Epigallocatechin contributes a low of 2.44 mg/L. Dihydrocalcone derivatives make up 6% of total polyphenols and Flavonols represent 3% of the total polyphenol amount. Anthocyanins are present in mostly undetectable values, contributing to 0.6% of the total polyphenols present. This is expected because anthocyanins are concentrated in the skin of the apple and are usually not water soluble (Wolfe, 2003). A study conducted in Trentino, Italy presents Flavanols (71-90%), hydroxycinnamates (4-18%), flavonols (1-11%), dihydrochalcones (2-6%), and anthocyanins (1-3%). The discrepancies noted in the Trentino, Italy study can be contributed to the preparation of juices, which was documented as using an acetone/water extraction method compared to Study 1 and Study 2's investigation of purely water soluble substances. As mentioned earlier, investigating water soluble compounds more accurately represents the presence of bioavailable substances.

Analysis of mineral and ion content

Mineral content of juices investigated totaled 366.79 mg/L, seen in **Table 11**. Sulfate represented 96% of total anions and potassium represented 97% of total cations present in juices. Linear regression correlation analysis between sulfate and potassium has a positive and strong correlation. This is suggestive that the main salt for apples is potassium sulfate. No previous literature can be found on this and further research should be done to determine the cause for this. F- is present in 74.81 mg/L. This represents the second highest concentration of anions in the apple samples. Mg^{+2} represents the second highest concentration of cations (31.01 mg/L).

Table 11: Mineral content (mg/L) of apple juices under study 2.

	Mean	SD	%CV	Minimum	Maximum	Range
F^-	74.81	39.73	53.11%	30.31	226.49	196.18
Cl^-	4.17	6.24	149.55%	0.00	43.24	43.24
NO_3^-	13.89	9.54	68.69%	0.00	65.97	65.97
SO_4^{2-}	1990.87	630.17	31.65%	763.09	3786.65	3023.56
Σ Anions	**2083.74**					
NH_4^+	2.50	2.62	104.72%	0.14	16.43	16.29
K^+	1530.80	513.04	33.51%	652.30	3460.41	2808.10
Mg^{+2}	31.01	10.72	34.57%	13.14	62.44	49.30
Ca^{+2}	19.73	9.21	46.67%	0.00	49.05	49.05
Σ Cations	**1584.15**					
Total Mineral Content	**3667.89**					

__**Analysis of single apples from selected varieties**__

To better characterize apples within cultivars, three apples from ten cultivars selected at random were further characterized. Then, three apples were randomly selected from each cultivar and were analyzed individually. Samples were prepared so that each apple juice originated from one apple and only includes the juice of that apple. This means that the juice studied originates from one apple and only includes the juice of that apple. This allows for a comparison of compositional characteristics within apple varieties and displays the variability in characteristics from apple to apple within the same cultivar.

Collectively, the maturity level of each apple studied can be identified as ripe, according to the FAO guidelines. As shown in **Table 12**, the mean Brix° value was 13.35 with a mean TA value of 1.18 (% as malic acid). Between each cultivar, malic acid averages a 27.98 % CV with a high of 79.86% and citric acid averages 30.10 % CV with a maximum of 70.80 %. Interestingly, both maleic acid and citric acid % CV represents a low of 0%. This indicates that some apple varieties, such as the Rheinischer Krummstiel and Berlepsch, display more stable characteristics than others. Additionally, a high % CV value in malic acid does not indicate a high % CV value in citric acid and vice versa.

Table 12: Ripeness parameters (Brix°, titratable acidity, malic acid, citric acid) of apple juice prepared from 10 apple varieties.

	Units	Mean	SD	%CV	Minimum	Maximum	Range
Brix°		13.35	1.95	14.60	9.60	16.60	7.00
TA	(% as malic)	1.18	0.52	43.80	0.67	2.68	2.01
Malic acid	(mg/L)	5282.37	4287.52	81.17	245.12	12586.29	12341.18
Citric acid	(mg/L)	102.40	118.73	115.95	5.16	464.47	459.30

When comparing the mixed apple juices and the averaged single apple juices, malic acid content differs as much as 65% (Liberty). Citric acid content varies even more with a high of 122% difference. Conversely, in the Rheinischer Krummstiel apple, citric acid varies by 1% and in the Scmidberger Renette, malic acid varies by 2%. These variations indicate inconsistent acid values between apples within the same cultivar grown in the same location. Supplementary information can be seen at the end of this section in **Tables 16-19**.

Within cultivars, the highest %CV value is 85.53% (Liberty) with a low of 6.60% (Maschschankzka) and a mean of 30.30%. TPC of individual apple juices is 1,794.8 mg/L compared to the average of the mixed apple juices (730.8 mg/L) with a 146% difference. This indicates a moderate variation between the cultivars themselves and a substantial difference in the range.

The FRAP values in the single apples are on average, much higher than the apple juices, comparing values of 29.6 mmol/L of single apples to 14.80 mmol/L of apple juices as seen in Table 3 and Table 7. Average ORAC values display the least variation between single apples (17.62 mmol/L) and the mixed juice (18.10 mmol/L).

Each apple variety had an average standard deviation of 2.98 for the FRAP with a minimum standard deviation of 0.4 in the Berlepsch apple and a maximum standard deviation of 9.3 in the Roter Boskoop. ORAC measurements displayed an average standard deviation of 5.46 with a minimum standard deviation of 0.58 in the Berlepsch apple and a maximum standard deviation of 25.73 in the Roter Boskoop. Interestingly, the apple with the lowest standard deviation is the Berlepsch apple and the highest standard deviation is shown in the Kanada Renette. The consistently low standard deviation for the Berlepsch apple could represent a variety with the most stable characteristics. The high standard deviation of TPC values indicates a large consistency in TPC from apple to apple within the same variety. This can be viewed in **Table 13.**

Table 13: Total phenolic content (TPC) and antioxidant capacities of individual apples under study.

	Units	Mean	SD	%CV	Minimum	Maximum	Range
TPC	(mg/L)	1794.8	1313.93	73.21	6.63	4524.09	4517.46
FRAP	(mmol/L)	29.6	15.91	53.79	10.00	55.79	45.79
ORAC	(mmol/L)	17.62	10.65	60.46	5.83	62.47	56.64

Below is the single polyphenol content of the individual's apples under study. There are a total of 301 hydroxycinnamic acids, 14 dihydrochalcone derivates, and 151 flavan-3-ols. Flavonols summed up to 6 and anthocyanins were undetected. This can be reviewed in **Table 14.**

The mineral content of apple juices closely represents the mixed juice analysis. This is seen in **Table 15.** Similar mineral content between apples is indication of a consistent parameter represented in sample juices. **Supplementary Tables 5-10** provide relevant information and show the averages between the averaged values of the mixed juices versus the averaged cultivars in triplicate values.

Table 14: Single polyphenol content (mg/L) of individual apples under study.

	Mean	**SD**	**%CV**	**Minimum**	**Maximum**	**Range**
Chlorogenic acid	288.38	131.32	45.54	71.94	601.19	529.25
Caffeic acid	6.22	3.83	61.61	0.00	12.88	12.88
4-*p*-Coumaroylquinic acid	6.56	7.29	111.15	0.10	24.72	24.62
Σ Hydroxycinnamic acids	**301.16**					
Phloretin-2'-*O*-xyloglucoside	5.44	3.96	72.85	1.14	17.90	16.76
Phloridzin	8.46	5.19	61.36	2.65	22.40	19.75
Σ Dihydrochalcone derivates	13.90					
Procyanidin B1	11.59	9.65	83.27	1.99	41.76	39.77
Procyanidin B2	85.95	50.72	59.01	11.39	200.28	188.89
(-)-Epicatechin	46.23	26.89	58.17	4.73	104.73	100.00
Epigallocatechin	2.77	1.93	69.73	0.97	7.92	6.95

Epichatechingallate	5.08	7.73	152.15	0.28	33.33	33.05
Σ Flavan-3-ols	**151.62**					
Quercetin-3-*O*-galactoside	1.21	1.39	114.69	0.16	7.19	7.03
Quercetin-3-*O*-xyloside	2.80	2.86	102.26	0.32	12.14	11.82
Quercetin-3-*O*-rhamnoside	1.08	0.74	68.88	0.00	3.19	3.19
Quercetin	1.35	1.09	81.00	0.16	5.91	5.75
Quercetin-3-*O*-rutinoside	0.14	0.24	168.51	0.00	1.28	1.28
Σ Flavonols	**6.58**					
Σ Anthocyanins	**n.d.**	n.d.	n.d.	n.d.	n.d.	n.d.
Total polyphenol amount (HPLC)	**473.27**					

n.d., not detectable; +, < limit of quantitation

Table 15: Mineral content of apple juices (individual) under study.

	Mean	SD	%CV	Minimum	Maximum	Range
Cl^-	15.4	2.75	1779.8	2.5	122.9	120.4
NO_3^-	18.3	2.69	1474.3	n.d.	135.9	135.9
SO_4^{2-}	25.1	1.18	469.3	9.2	53.7	44.5
Σ Anions	**378.57**					
Na^+	0.4	0.02	577.1	0.1	0.9	0.7
NH_4^+	1.4	0.19	1359.8	0.2	10.1	9.8
K^+	1798.9	40.26	223.8	1086.9	2649.6	1562.7
Mg^{+2}	27.7	1.66	601.0	10.9	81.9	71.0
Ca^{+2}	29.5	2.10	711.7	8.5	107.1	98.6
Σ Cations	**1857.8**					
Total Mineral Content	**5643.5**					

Table 16: % CV values are derived from comparison of three randomly selected apples per cultivar.

Cultivar	Brix	TA	TPC	ORAC	FRAP	Maleic acid	Citric acid
Roter Boskoop	8.5%	45.4%	70.5%	35.6%	79.7%	23.5%	50.2%
Schmidberger Renette	4.7%	1.7%	43.9%	96.2%	70.6%	2.1%	14.3%
Oldenwälder	8.8%	45.4%	53.5%	15.2%	81.1%	42.1%	20.5%
Deans Küchenapfel	1.3%	34.4%	68.2%	49.5%	81.2%	6.4%	70.6%
Kanada Renette	10.3%	26.0%	56.0%	53.5%	78.4%	34.4%	101.1%
Rheinischer Krummstiel	5.4%	19.9%	50.0%	60.3%	46.7%	19.9%	0.8%
Rewena	3.5%	3.0%	54.4%	94.4%	12.3%	53.8%	121.9%
Berlepsch	18.3%	33.2%	5.0%	570.2%	12.7%	11.9%	112.6%
Maschanzker	10.4%	31.3%	88.8%	64.8%	9.9%	11.3%	67.7%
Liberty	6.0%	19.9%	27.8%	28.5%	31.7%	65.2%	112.6%

Table 17: % CV values are derived from comparison of three randomly selected apples per cultivar.

Cultivar	K+	Mg2+	Ca^{2+}	CL-
Roter Boskoop	27.3%	37.1%	60.7%	71.1%
Schmidberger Renette	5.0%	2.8%	7.6%	84.4%

Oldenwälder	4.5%	0.9%	45.5%	100.0%
Deans Küchenapfel	35.3%	15.7%	38.9%	96.8%
Kanada Renette	41.5%	23.3%	51.1%	69.8%
Rheinischer Krummstiel	41.4%	14.1%	44.9%	61.5%
Rewena	24.5%	65.8%	86.9%	49.8%
Berlepsch	3.7%	48.8%	23.5%	87.1%
Maschanzker	44.3%	28.3%	53.8%	100.0%
Liberty	12.0%	146.8%	39.6%	38.4%

Table 18: % CV values are derived from comparison of three randomly selected apples per cultivar.

Cultivar	**QU-3-O-rh**	**QU-3-O-x**	**QU-3-O-g**	**QU**	**CGA**	**CAFF**	
Roter Boskoop	76.9%	25.0%	740.0%	312.5%	20.0%	n.d.	4.9%
Schmidberger Renette	20.0%	387.5%	1100.0%	275.0%	32.5%	20.7%	11.2%
Oldenwälder	52.6%	8.7%	5.4%	40.0%	26.7%	49.1%	22.4%
Deans Küchenapfel	83.3%	23.4%	35.7%	57.1%	83.9%	68.3%	17.9%

Table 19: % CV values are derived from comparison of three randomly selected apples per cultivar.

Cultivar	**Phloz**	**Phloz-2-O**	**Epi**	**ECG**	**EGC**	**PCB1**	**PCB2**
Roter Boskoop	15.6%	**3.2%**	78.3%	200.0%	226.1%	91.3%	54.0%
Schmidberger Renette	25.1%	**98.0%**	80.4%	16.7%	21.1%	88.5%	74.9%
Oldenwälder	72.7%	**91.4%**	69.4%	105.7%	75.5%	60.5%	71.0%
Deans Küchenapfel	79.6%	**13.0%**	96.6%	350.0%	50.0%	77.3%	93.2%
Kanada Renette	87.8%	**82.8%**	99.4%	500.0%	41.7%	83.9%	97.6%
Rheinischer Krummstiel	55.0%	**63.6%**	98.5%	26.3%	237.5%	65.5%	93.6%
Rewena	91.5%	**91.1%**	98.3%	25.0%	93.8%	100.0%	98.5%
Berlepsch	91.6%	**57.4%**	96.3%	88.9%	100.0%	90.0%	98.1%
Maschanzker	64.1%	**60.4%**	61.1%	77.3%	25.0%	10.9%	67.5%
Liberty	100.0%	**100.0%**	100.0%	100.0%	100.0%	100.0%	100.0%

Comparison of Apple Cultivars Grown in two different regions of Austria

Identical cultivars from Study 1 and Study 2. This comparison represents the variations between the same cultivars grown in different geographical regions. The apples were cultivated under different climates, solid composition and water profiles. Cultivars investigated can be seen in **Table 2**.

Nineteen apple varieties were present in both study cohorts. Each variety selected was cultivated in Tulln/Lower Austria and Eferding/Upper Austria. When comparing same species apples, Brix° values averaged a difference of 7% with a high of 17% variation (Geheimrat Dr. Oldenburg, Hausapfel, Jonathan) and a low of 0% variation (Odenwälder). All associated values can be found on **Supplemental Table 4**. This signifies that the apples being compared are all in the ripe stage.

However, the ripeness of apples were in some cases as different as 142% and as low as 1%. These variations can account for different levels of composition when characterizing cultivars. This is because the contents of fruits are known to vary with different levels of maturity. Total phenolic content and antioxidant capacity values measured in this study differed from the first study by an average of (2%). Citric and maleic acid values varied significantly between the two studies, however, citric and maleic acid values also varied significantly between single apples of the species grown in the same geographical conditions. Therefore, differences noted in Study 1 and Study 2 cannot be identified as a result of cultivation in a different geographical region. Further studies must be done to determine the definite cause of noted differences.

Maleic acid, citric acid and total acid contents displayed outstanding variations between the same apples grown within different geographical regions averaging 134%, 724% and 134% differences respectively. Maleic Acid displayed a maximum of 1370% difference in the Plankenapfel with a minimum in the Roter Herbstcalvill apple. Citric acid displayed a maximum percent difference of 11463% in the Plankenapfel and a minimum of 0% in the Roter Passamaner apple. Total Acid measured showed the Plankenapfel having the greatest variation (1385%) and the Roter Herbstcalvill having the minimum variation of 5%. Large differences in acid contents are expected because the natural degradation and ripening process individual apples experience after harvesting. Apples cultivated in the same geographical region during the same year experienced maximums of 80% CV with a low of 0.29% CV. The apple cultivars with the lowest CV are classified as cooking apples, which are characterized by having high acid contents. The higher the acid content in an apple, the less susceptible an apple is to quick deterioration of acid to sugars in the overall maturity cycle of an apple. Therefore, noted differences in acid content do not occur primarily because of climate, year or geographical region.

Interestingly, titratable acidity had as low as a fourfold decrease in CV (77%). The minimum and maximum differences in TA are from 0% to 100% in Boikenapfel and Retina respectively. Interesting the Boikenapfel is known as a resistant cultivar- being able to flourish in any region as well as any soil type. The Retina apple has a short shelf life which is characterized by a quick degradation rate. Therefore, this species is more sensitive to the storage temperature, storage time and level of maturation when picked. Consequently, it is expected to see larger CV differences.

The second studies included FRAP measurements instead of TEAC measurements to identify antioxidant capacity. This is because FRAP measurements are known to be more reliable and consistent than TEAC measurements. Therefore, there are no FRAP values to compare from the varying region and years.

However, ORAC measurements, also identifying antioxidant capacities within apples, can be compared. ORAC values between the two studies experienced a mean of 70% variation with a maximum of 27% (Plankenapfel) and a minimum of 4% (Pilot). Similarly the Roter Boskoop, Schimberger Renette Plankenapfrel and Deans Kuchinapfel displayed variations of 5%, 6% and 16% variation. Interestingly, these apples are all classified as a good cooking apple, which is characterized by a high acid content and sour taste.

TCP displayed an average of 87% difference with a high of 763% (Boikenapfel) and a low of 4% (Liberty). When the Boilken apple is removed as an outlier the average drops to 49% variation. Interestingly enough, TPC is a relatively consistent, dependable and reliable study. Therefore, these variations can be due to soil composition cultivation, weather differences and etc.

Polyphenols examined displayed high levels of variation. Non detectable levels of anticyanins were displayed. This is expected because anticyanin levels are concentrated in the peel of the apple. Dihydrocalcones averaged a 79% difference. Flavanols displayed a 70% averaged difference between the five flavanols examined. Hydroxycinnamic acids experienced a 74% difference averaged between the three hydoxycinnamic acids examined. Flavonols showed outstanding differences- averaging 22%. However, the compound QU-30-g shows a difference of 616%. Investigation needs to be done on the validity of research as well as the consistency in respective measurements.

Interestingly, a study published compared ORAC, anthocyanin, and total phenolic content between blueberries grown in Oregon, Michigan, and New Jersey. No significant differences were noted between measurement values and growing location (Prior, 1998). This finding coincides with small apple juice variations between the two sites in Austria.

Further identification and characterization of apple cultivars should be researched in order to determine cultivars with the highest health benefits. Ways to increase polyphenol content and antioxidant capacity in commonly sold or easily mass produced apple varieties should be investigated. A study conducted in Slovenia researched what a decrease in crop load on Jonagold apples would do. The results in the study state that both the quality of the fruit and the polyphenol content increased, noting single polyphenol increases as large as 82%. (Stopar, 2002).

Conclusion

In conclusion these studies help provide insight to what the human population is consuming. Especially as apples are considered important contributors to human health, there is potential to maximize health benefits derived from apple consumption by choosing the right variety.

When these apple varieties were compared, it was noted that some prevalent market apples, like Gala, don't provide very high levels of antioxidants compared to other species. In order to combat modern diseases, prevalent in both developed and under developed countries, healthy lifestyles must be taken into consideration. For the context of this discussion, a healthy lifestyle is defined by a proper diet. If foods with preventative capabilities were staple parts of a healthy lifestyle, it's possible to see a decrease in diseases such as diabetes and heart disease over the human population. Industry and proper marketing techniques need to be taken into consideration to come close to providing individuals with the most health benefiting options.

Apples and other foods that should be marketed more are the ones containing the highest amount of polyphenols and display the greatest level of bioavailibity. This is because if the human body cannot access the polyphenols, it cannot provide said health benefits. In conclusion, the characterization and composition of foods, such as apples, is important. With an increased knowledge on composition and availability, the human population can change industry behaviours and provide healthier options for the human population as a whole.

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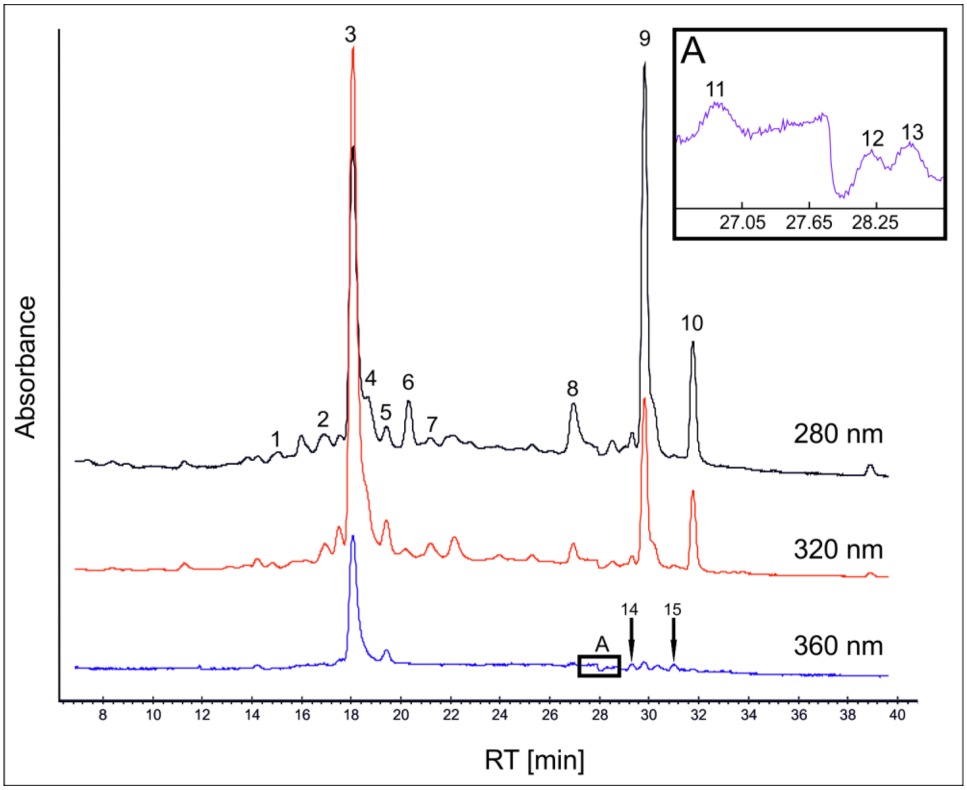
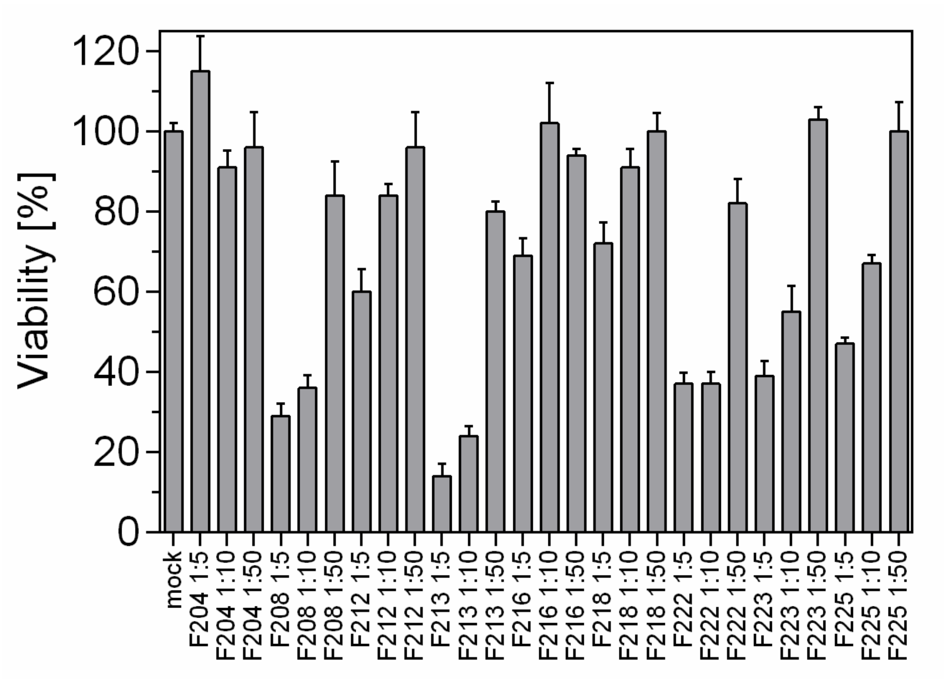
Supplementary Information



Supplementary Figure 1: HPLC elution profile of a representative apple juice sample detected at 280 nm, 320 nm and 360 nm, respectively. 1, Procyanidin B1; 2, Epigallocatechin; 3, Chlorogenic acid; 4, Procyanidin B2; 5, Caffeic acid; 6, Epicatechin; 7, 4-p-Coumarylquinic acid; 8, Epicatechingallate; 9, Phloretin 2'-*O*-xylosyl-glucoside; 10, Phloridzin; 11, Quercetin 3-*O*-rutinoside; 12, Quercetin 3-*O*-galactoside; 13, Quercetin; 14, Quercetin 3-*O*-xyloside; 15, Quercetin 3-*O*-rhamnoside.



Supplementary Figure 2: Influence of selected apple juice samples with varying total phenolic content (TPC) on HuH-7 cell viability. HuH-7 cells were grown to 90% confluency in 96-well plates and incubated with apple juice diluted in cell culture medium at indicated concentrations for 6 hours. Cell viability is given in percent in comparison to a non-treated sample. Error bars are based on the standard error of the mean (n = 4).

Supplementary Table 1: Study 1, 67 Organically grown Apple Cultivars.

Cultivar	TPC [mg/L]	ORAC [mmol/L]	TEAC [mmol/L]	Brix [°]	K^+ [mg/L]	Mg^{2+} [mg/L]	Ca^{2+} [mg/L]	Cu^{2+} [mg/L]	Mn^{2+} [mg/L]	Fe^{2+} [mg/L]	PO_4^{3-} [mg/L]	TA [% of malic]	Malic acid [mg/L]	Citric acid [mg/L]
Rheinischer Krummstiel	975.4 ± 69.2	15.7 ± 0.9	4.32 ± 0.13	12.0	963.9 ± 2.3	29.5 ± 0.8	20.7 ± 0.1	264.4 ± 1.1	617.9 ± 1.2	n.m.	131.0 ± 1.6	0.67	8598.1	57.5
Retina	252.7 ± 18.8	7.2 ± 0.5	1.44 ± 0.11	11.0	620.4 ± 6.8	19.8 ± 0.4	6.7 ± 0.3	n.m.	n.m.	n.m.	n.m.	0.27	3833.4	173.8
Maschanzker	1340.4 ± 66.4	26.0 ± 1.0	5.48 ± 0.51	14.0	1409.1 ± 5.4	60.3 ± 1.2	53.7 ± 0.6	n.m.	n.m.	n.m.	n.m.	1.01	11598.1	173.8
Carpetin (kleine Weinrenette)	903.1 ± 9.0	22.0 ± 1.7	2.13 ± 0.83	12.0	1976.8 ± 0.6	69.8 ± 1.3	51.1 ± 0.8	n.m.	n.m.	n.m.	n.m.	0.74	7892.2	173.8
Sommerrambour	672.7 ± 24.9	15.9 ± 0.8	3.13 ± 1.54	13.0	1361.7 ± 2.5	47.5 ± 0.9	18.8 ± 0.8	152.9 ± 1.5	562.8 ± 1.2	n.m.	266.5 ± 0.8	1.07	13539.2	522.6
Dülmäner Rosenapfel	1008.7 ± 13.7	25.9 ± 3.6	4.99 ± 0.28	12.9	1080.4 ± 8.1	30.9 ± 0.8	21.4 ± 0.6	n.m.	n.m.	n.m.	n.m.	0.60	8774.5	348.2
Pilot	1266.8 ± 26.2	25.1 ± 0.5	4.12 ± 0.30	14.0	1371.1 ± 6.3	53.8 ± 0.6	36.2 ± 1.2	318.6 ± 0.7	655.5 ± 1.7	n.m.	120.5 ± 1.3	0.87	3892.2	57.5
Roter Passamaner	819.2 ± 44.6	32.1 ± 0.1	2.89 ± 0.84	13.0	977.1 ± 7.1	39.4 ± 0.6	21.2 ± 0.4	n.m.	n.m.	n.m.	n.m.	0.60	2245.1	115.6
Schmidberger Renette	357.7 ± 16.5	10.9 ± 1.7	1.05 ± 0.67	11.9	961.3 ± 8.1	33.7 ± 0.5	16.1 ± 1.3	n.m.	n.m.	n.m.	n.m.	0.74	9656.9	173.8
Gelber Edelapfel	1279.0 ± 47.5	23.6 ± 1.9	2.09 ± 0.77	15.9	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	0.81	8186.3	173.8
Bismarck	297.1 ± 5.9	10.7 ± 0.4	2.15 ± 0.60	13.0	1284.2 ± 1.7	39.5 ± 0.3	10.4 ± 0.3	n.m.	n.m.	n.m.	n.m.	1.28	4598.1	57.5
Schieblers Taubenapfel	578.0 ± 20.1	14.6 ± 0.4	1.87 ± 0.30	11.0	1202.2 ± 6.9	41.3 ± 0.5	24.5 ± 0.2	200.6 ± 0.9	586.1 ± 2.5	n.m.	190.9 ± 0.3	0.87	8068.6	173.8
Liberty	634.2 ± 15.8	16.4 ± 0.5	3.48 ± 1.15	12.0	1346.4 ± 9.8	40.4 ± 2.1	17.8 ± 0.3	n.m.	n.m.	n.m.	n.m.	0.67	3598.1	28.4
Alkmene	676.7 ± 19.0	14.2 ± 0.2	1.91 ± 0.20	16.5	1204.6 ± 4.3	38.4 ± 0.9	16.6 ± 0.2	n.m.	n.m.	n.m.	n.m.	0.94	12009.8	115.6
Jonathan	1493.1 ± 46.7	26.1 ± 0.5	4.15 ± 1.50	14.9	1752.0 ± 1.9	64.4 ± 1.2	34.9 ± 0.6	n.m.	n.m.	n.m.	n.m.	1.07	11774.5	115.6
Herrenapfel	1009.2 ± 70.4	25.8 ± 0.2	5.99 ± 1.07	17.0	856.0 ± 8.7	33.8 ± 2.1	18.5 ± 1.2	530.3 ± 2.1	661.5 ± 3.3	n.m.	170.6 ± 3.2	1.61	17245.1	348.2
Spitzling	939.4 ± 63.4	20.9 ± 2.5	2.52 ± 0.22	14.5	995.9 ± 10.1	48.4 ± 1.2	43.4 ± 1.3	n.m.	n.m.	n.m.	n.m.	0.74	9598.1	115.6
Hausapfel	410.6 ± 1.8	16.8 ± 0.6	3.61 ± 0.22	14.5	663.8 ± 7.7	32.3 ± 0.2	32.9 ± 0.9	n.m.	n.m.	n.m.	n.m.	0.74	10127.5	115.6
Spitzapfel	1092.8 ± 9.3	21.2 ± 2.2	4.11 ± 0.29	12.9	724.0 ± 4.6	27.5 ± 0.6	24.8 ± 0.5	n.m.	n.m.	n.m.	n.m.	0.81	2951.0	57.5
Graue Herbstrenette	624.1 ± 17.5	13.2 ±	2.61 ±	17.0	1073.7 ±	42.3 ±	25.2 ± 0.1	n.m.	n.m.	n.m.	n.m.	0.54	6421.6	173.8

		0.05	0.56		8.1	0.9								
Harberts Renette	2242.3 ± 75.8	58.9 ± 2.2	6.38 ± 1.83	18.9	636.7 ± 7.1	31.0 ± 1.3	30.6 ± 1.5	n.m.	n.m.	n.m.	n.m.	1.21	16245.1	173.8
Lesans Kalvill	819.7 ± 13.6	15.2 ± 0.2	3.46 ± 0.77	14.9	1162.6 ± 7.4	31.4 ± 0.6	10.7 ± 0.1	289.4 ± 1.8	542.3 ± 3.6	n.m.	278.3 ± 0.9	1.07	8833.4	57.5
Samareiner Rosmarien	846.2 ± 35.7	35.7 ± 0.7	5.80 ± 0.84	15.0	640.9 ± 6.1	24.9 ± 0.9	25.1 ± 0.3	n.m.	n.m.	n.m.	n.m.	0.47	127.5	28.4
Berneder	296.3 ± 47.2	14.0 ± 0.7	2.64 ± 0.20	13.0	949.4 ± 2.5	35.8 ± 0.8	18.9 ± 0.1	312.4 ± 0.8	586.1 ± 1	n.m.	192.7 ± 7.8	1.07	10951.0	115.6
Roter Boskoop	1135.6 ± 1.2	21.3 ± 0.4	4.43 ± 0.33	13.5	650.9 ± 6.1	26.7 ± 0.7	23.7 ± 0.1	332.5 ± 1.9	679.0 ± 2.1	n.m.	255.7 ± 3.9	0.74	7362.8	57.5
Goldrenette Freiherr v. Berlepsch	524.2 ± 63.6	16.8 ± 0.1	3.91 ± 0.12	14.9	1434.3 ± 6.3	42.7 ± 1.2	30.3 ± 0.6	232.4 ± 1.6	552.0 ± 3.1	n.m.	212.8 ± 1.4	1.14	13951.0	173.8
Florianaer Rosmarin	1049.5 ± 51.4	17.3 ± 0.5	2.67 ± 0.69	14.9	859.8 ± 5.4	29.3 ± 1.2	12.4 ± 0.1	n.m.	n.m.	n.m.	n.m.	0.74	7715.7	115.6
Boikenapfel	103.2 ± 1.1	8.4 ± 0.3	1.27 ± 0.04	14.0	845.4 ± 7.1	28.1 ± 2.1	17.7 ± 0.4	n.m.	n.m.	n.m.	240.1 ± 0.6	0.94	10951.0	115.6
Roter Stettinger	1511.8 ± 99.1	49.1 ± 3.4	3.80 ± 0.56	13.5	981.0 ± 8.2	30.4 ± 0.5	11.1 ± 0.6	n.m.	n.m.	n.m.	n.m.	0.94	10715.7	115.6
Kammerapfel	800.3 ± 6.3	14.7 ± 0.4	2.56 ± 0.29	13.9	775.9 ± 9.2	25.1 ± 1.4	11.1 ± 0.1	n.m.	n.m.	n.m.	n.m.	0.81	6951.0	173.8
Weißer Winter-Taffetapfel	788.7 ± 55.1	29.8 ± 2.9	4.02 ± 0.53	13.0	1122.1 ± 1.4	31.6 ± 1.3	28.6 ± 0.3	152.4 ± 2.2	612.9 ± 1.6	n.m.	119.4 ± 0.8	0.47	303.9	28.4
Odenwälder	2275.6 ± 92.4	27.7 ± 0.7	6.27 ± 3.16	12.0	n.m.	n.m.	n.m.	209.6 ± 2	549.3 ± 2.6	n.m.	324.4 ± 5	1.21	8245.1	115.6
Damason Renette	592.1 ± 23.9	20.4 ± 0.1	4.60 ± 0.38	13.5	1443.8 ± 5.2	49.9 ± 0.8	29.6 ± 0.6	n.m.	n.m.	n.m.	n.m.	0.94	13480.4	173.8
Champagner Renette	1128.0 ± 35.8	16.7 ± 1.5	5.29 ± 0.77	17.5	1624.7 ± 1.6	34.8 ± 0.7	20.4 ± 0.2	n.m.	n.m.	n.m.	n.m.	1.34	12480.4	57.5
Hauxapfel	655.3 ± 46.9	11.2 ± 0.3	2.80 ± 0.27	15.5	1179.9 ± 7.5	41.7 ± 1.4	23.0 ± 0.1	n.m.	n.m.	n.m.	224.4 ± 3.5	0.47	421.6	28.4
Glockenapfel	1028.7 ± 45.2	19.7 ± 0.6	2.90 ± 1.12	11.0	695.4 ± 1.3	33.8 ± 1.2	27.2 ± 0.2	157.4 ± 1.6	588.6 ± 3.6	n.m.	241.9 ± 3.7	1.14	3715.7	28.4
Zuccalmaglios Renette	2264.6 ± 21.0	29.1 ± 1.9	6.03 ± 2.72	12.5	1298.4 ± 2.3	37.6 ± 1.0	23.6 ± 0.6	n.m.	n.m.	n.m.	n.m.	1.34	15774.5	290.0
Roter James Grieve	503.7 ± 7.5	15.1 ± 1.3	3.58 ± 0.12	14.0	1200.7 ± 4.5	38.6 ± 0.6	17.9 ± 0.9	n.m.	n.m.	n.m.	n.m.	0.87	10774.5	406.3
Weißer Passamaner	401.7 ± 15.1	37.8 ± 0.4	2.21 ± 0.01	14.5	832.5 ± 2.1	37.3 ± 0.9	26.3 ± 0.7	n.m.	n.m.	n.m.	n.m.	1.21	14127.5	406.3
Plankenapfel	671.2 ± 39.2	11.5 ± 0.6	2.51 ± 0.03	13.0	1112.7 ± 8.5	44.4 ± 1.2	36.6 ± 0.1	n.m.	n.m.	n.m.	n.m.	0.67	656.9	28.4
Cultivar	TPC [mg/L]	ORAC [mmol/L]	TEAC [mmol/L]	Brix [°]	K^+ [mg/L]	Mg^{2+} [mg/L]	Ca^{2+} [mg/L]	Cu^{2+} [mg/L]	Mn^{2+} [mg/L]	Fe^{2+} [mg/L]	PO_4^{3-} [mg/L]	TA [% of malic]	Malic acid [mg/L]	Citric acid [mg/L]

Riesenboikenapfel	828.5 ± 2.2	18.5 ± 1.5	3.23 ± 0.10	16.0	1009.6 ± 9.1	39.9 ± 0.6	30.1 ± 0.3	n.m.	n.m.	n.m.	205.5 ± 2.2	0.67	362.8	28.4
Ananasrenette	708.8 ± 9.6	23.7 ± 5.2	2.50 ± 1.24	11.5	711.5 ± 7.1	26.1 ± 0.7	23.0 ± 0.3	n.m.	n.m.	n.m.	n.m.	0.47	6656.9	115.6
Glasapfel	254.7 ± 28.1	7.1 ± 0.2	2.27 ± 0.95	12.0	932.7 ± 8.1	20.3 ± 1.1	45.5 ± 0.4	n.m.	n.m.	n.m.	n.m.	1.14	1127.5	28.4
Steirischer Maschanzker	975.0 ± 18.7	18.8 ± 0.9	3.45 ± 0.11	10.9	1411.8 ± 7.8	14.6 ± 1.2	28.6 ± 0.5	572.2 ± 2.5	150.9 ± 1.4	n.m.	219.3 ± 1.3	0.60	5362.8	57.5
Pinova	428.6 ± 14.3	7.1 ± 0.4	1.26 ± 0.19	14.0	1153.4 ± 7.6	12.6 ± 0.3	21.3 ± 0.8	n.m.	n.m.	n.m.	n.m.	0.67	9480.4	231.9
Roter Griesapfel	1137.0 ± 38.9	36.0 ± 4.7	3.78 ± 0.23	13.9	760.0 ± 2.6	9.9 ± 0.5	45.2 ± 0.1	n.m.	n.m.	n.m.	n.m.	0.81	8421.6	231.9
Gelber Bellefleur	475.3 ± 9.9	8.4 ± 0.3	2.35 ± 0.17	13.0	1664.3 ± 6.3	13.2 ± 0.1	15.9 ± 1.0	403.2 ± 1.7	117.1 ± 1	n.m.	332.2 ± 5.9	0.60	10186.3	115.6
Rewena	221.0 ± 6.1	10.3 ± 1.3	1.14 ± 0.13	12.0	1137.9 ± 6.5	13.2 ± 0.2	43.8 ± 0.7	439.9 ± 0.8	248.2 ± 0.5	n.m.	162.0 ± 1.1	0.60	9127.5	115.6
Piros	274.9 ± 10.7	7.8 ± 0.4	1.45 ± 0.33	14.9	1269.9 ± 8.2	12.5 ± 0.6	20.5 ± 0.7	n.m.	n.m.	n.m.	n.m.	0.54	6009.8	115.6
Schöner v. Wiltshire	610.4 ± 23.5	18.7 ± 0.2	3.47 ± 0.64	13.0	1226.1 ± 4.5	10.8 ± 0.7	14.9 ± 0.6	408.5 ± 2.1	105.6 ± 1.4	n.m.	190.0 ± 2.4	0.94	5598.1	115.6
Prinzenapfel	671.3 ± 77.9	23.4 ± 0.2	5.04 ± 0.20	12.9	927.4 ± 2.8	9.3 ± 0.1	23.9 ± 0.1	345.4 ± 0.8	136.4 ± 1.3	n.m.	142.1 ± 1.2	1.21	16421.6	115.6
Kleiner Feiner	622.6 ± 5.0	31.2 ± 1.8	2.65 ± 0.23	12.0	980.8 ± 7.5	10.9 ± 0.8	28.5 ± 1.2	392.8 ± 1.3	131.8 ± 1.1	n.m.	120.5 ± 1.3	0.74	8951.0	173,8
Geheimrat Oldenburg	989.8 ± 24	32.2 ± 4.6	3.36 ± 0.13	12.0	1026.3 ± 8.7	9.4 ± 0.5	16.1 ± 0.9	497.7 ± 3.5	91.6 ± 0.7	n.m.	283.7 ± 2.5	1.95	16009.8	173,8
Deans Küchenapfel	950.2 ± 40.4	22.7 ± 0.7	5.14 ± 0.33	12.5	1155.0 ± 9.1	8.4 ± 0.7	11.3 ± 0.3	384.6 ± 1.6	136.8 ± 0.5	n.m.	226.8 ± 1.6	0.54	2362.8	57,5
Zabergau Renette	329.8 ± 30.2	28.7 ± 2.4	1.87 ± 0.36	13.0	1280.4 ± 6.7	12.3 ± 0.7	20.0 ± 0.8	n.m.	n.m.	n.m.	n.m.	0.47	362.8	n.d.
Kanada Renette	700.7 ± 4.5	9.6 ± 0.2	3.42 ± 0.29	14.0	1449.8 ± 6.2	13.1 ± 0.3	15.4 ± 0.6	n.m.	n.m.	n.m.	335.3 ± 5.6	0.60	8362.8	115,6
Winter-Goldparmäne	1869.9 ± 36	28.7 ± 3.2	6.04 ± 1.62	15.0	1255.4 ± 5.2	12.8 ± 0.6	29.5 ± 1.2	381.1 ± 1.9	99.3 ± 1.2	n.m.	309.1 ± 1.2	0.94	8892.2	173,8
Grüner Boskoop	876.2 ± 37.9	12.3 ± 0.6	3.94 ± 0.75	11.9	2064.0 ± 6.8	18.8 ± 0.8	26.4 ± 1.1	383.0 ± 1.7	306.9 ± 1.4	n.m.	310.4 ± 1.6	0.47	5833.4	173,8
Relinda	504.1 ± 13.6	12.2 ± 0.4	1.93 ± 0.35	16.5	1188.9 ± 7.8	15.3 ± 0.9	30.0 ± 0.9	435.5 ± 1.3	225.0 ± 1.3	n.m.	144.9 ± 1.1	0.81	10480.4	115,6
Pom. Kongreß	1315.0 ± 49.8	16.4 ± 0.6	7.78 ± 0.71	12.9	1278.8 ± 4.6	12.0 ± 0.4	10.6 ± 0.2	497.6 ± 1.8	150.6 ± 0.9	n.m.	127.3 ± 2.4	0.94	11833.4	173,8
Fasslapfel	597.1 ± 37.2	22 ± 0.4	4.28 ± 0.00	14.0	852.3 ± 8.3	9.4 ± 0.7	24.9 ± 0.5	237.6 ± 1.6	608.37 ± 5.1	n.m.	118.5 ± 0.5	0.67	8715.7	115,6
Royal Gala (F204)	185.8 ± 4.7	3.5 ± 0.6	0.90 ± 0.02	10.9	790.5 ± 3.5	25.1 ± 0.2	17.4 ± 0.05	318.0 ± 3.0	94.0 ± 3.0	145.0 ± 15.0	208.6 ± 1.3	0.34	3068.6	57.5

Winterzitrone (F208)	1029.6 ± 38.1	21.9 ± 2.2	3.83 ± 0.12	12.9	686.0 ± 1	22.8 ± 0.1	21.7 ± 2.0	373.5 ± 7.5	94.0 ± 1.0	245.0 ± 5.0	169.8 ± 0.6	0.67	3833.4	57.5
Weißer Winterkalvill (F210)	728.6 ± 43.0	10.9 ± 0.8	1.76 ± 0.15	13.0	1258.0 ± 10	29.6 ± 0.2	13.4 ± 0.1	368.5 ± 38.5	118.5 ± 1.5	525.0 ± 55.0	278.2 ± 1.6	0.67	1833.4	28.4
Stäubli 2 (F212)	318.5 ± 18.1	9.3 ± 0.3	1.34 ± 0.20	10.0	696.0 ± 15	26.2 ± 0.4	13.5 ± 0.4	267.0 ± 20.0	152.0 ± 1.0	225.0 ± 125.0	158.2 ± 3.3	0.81	3480.4	28.4
Christkindler (F213)	1022.0 ± 3.5	21.9 ± 0.5	5.25 ± 0.16	12.0	1389.0 ± 9	35.8 ± 0.2	17.6 ± 0.2	355.0 ± 2.0	179.0 ± 1.0	195.0 ± 35.0	260.7 ± 1.9	1.07	9480.4	57.5
Freyperg (F215)	370.3 ± 14.0	6.6 ± 0.5	1.50 ± 0.16	14.5	1180.0 ± 1	37.2 ± 0.2	15.3 ± 0.2	552.5 ± 17.5	120.5 ± 1.5	170.0 ± 10.0	338.4 ± 0.3	0.47	3539.2	57.5
Topaz (F216)	257.4 ± 10.6	5.2 ± 0.2	1.10 ± 0.06	10.9	1037.5 ± 4.5	33.6 ± 0.1	19.3 ± 0.4	483.5 ± 5.5	190.0 ± 1.0	360.0 ± 60.0	261.9 ± 0.7	0.67	6068.6	57.5
Florina (F218)	224.2 ± 5.5	2.9 ± 0.6	0.85 ± 0.07	10.0	898.5 ± 8.5	28.1 ± 0.3	19.0 ± 0.2	549.0 ± 4.0	156.5 ± 4.5	185.0 ± 15.0	183.5 ± 1.0	0.40	3833.4	173.8
Wachsrenette (F219)	919.9 ± 21.1	17.2 ± 0.7	3.31 ± 0.14	12.5	970.5 ± 2.5	28.4 ± 0.2	11.9 ± 0.7	507.0 ± 3.0	106.5 ± 4.5	415.0 ± 15.0	236.3 ± 0.6	0.87	7539.2	57.5
Discovery (F221)	836.8 ± 12.7	23.5 ± 1.0	4.05 ± 0.21	8.0	812.0 ± 5	21.8 ± 0.1	17.6 ± 1.2	417.0 ± 38.0	59.5 ± 0.5	190.0 ± 60.0	166.3 ± 0.7	0.34	1539.2	28.4
Sponheimer Flurapfel (F222)	881.3 ± 4.2	16.3 ± 0.9	4.27 ± 0.65	10.0	1032.5 ± 2.5	35.1 ± 0.1	22.2 ± 0.0	432.0 ± 10.0	189.0 ± 7.0	220.0 ± 20.0	227.0 ± 0.7	0.60	3598.1	57.5
Roter von Siemonffi (F223)	1094.6 ± 30.2	21.6 ± 0.9	4.36 ± 0.54	11.0	701.5 ± 0.5	26.5 ± 0.2	18.4 ± 0.2	237.0 ± 4.0	121.5 ± 1.5	160.0 ± 10.0	160.5 ± 0.7	0.74	5362.8	57.5
Grüter Edelapfel (F225)	785.9 ± 14.3	12.7 ± 0.7	3.25 ± 0.26	9.9	772.5 ± 0.5	20.8 ± 0.2	14.5 ± 0.4	370.5 ± 0.5	77.0 ± 1.0	235.0 ± 5.0	151.4 ± 0.0	0.54	3127.5	57.5
Rheinischer Winterrambur (L200)	1087.4 ± 43.1	31.1 ± 1.3	4.45 ± 0.14	12.0	756.5 ± 3.5	29.8 ± 0.3	20.9 ± 1.3	343.5 ± 0.5	189.5 ± 3.5	305.0 ± 35.0	199.2 ± 3.3	0.47	4715.7	28.4
Dr. Seeligs Orangenpepping (L201)	527.8 ± 23.9	10.8 ± 1.1	1.32 ± 0.21	11.0	889.5 ± 1.5	33.7 ± 0.3	18.1 ± 0.1	386.5 ± 3.5	211.5 ± 6.5	670.0 ± 60.0	251.8 ± 1.3	0.67	1774.5	28.4
Von Zuccalmaglios Renette (L202)	1035.7 ± 64.0	26.3 ± 1.2	4.15 ± 0.31	14.0	1301.0 ± 10	38.2 ± 0.3	14.3 ± 0.9	208.5 ± 8.5	165.0 ± 2.0	260.0 ± 10.0	219.9 ± 0.7	0.60	2362.8	28.4
Mostzigeuner (L203)	1283.5 ± 66.0	21.6 ± 0.9	7.33 ± 0.74	11.0	1085.0 ± 21	35.0 ± 0.6	40.0 ± 0.7	303.0 ± 6.0	222.0 ± 1.0	130.0 ± 20.0	180.2 ± 3.0	1.14	12362.8	115.6
Blenheim (L205)	554.6 ± 6.5	12.2 ± 0.2	2.37 ± 0.23	12.0	982.5 ± 2.5	32.9 ± 0.1	12.9 ± 0.0	236.5 ± 3.5	187.5 ± 1.5	335.0 ± 5.0	261.3 ± 0.1	0.47	3186.3	57.5
Hallauer Maienapfel (L206)	863.8 ± 20.4	25.4 ± 1.8	4.57 ± 0.20	11.5	904.5 ± 9.5	27.6 ± 0.4	16.7 ± 0.7	177.5 ± 5.5	185.5 ± 5.5	225.0 ± 15.0	195.3 ± 1.2	0.60	5774.5	57.5
Seeländer Reinette (L207)	270.9 ± 18.7	11.4 ± 1.3	1.07 ± 0.12	12.5	870.5 ± 2.5	32.2 ± 0.0	22.7 ± 0.4	198.0 ± 2.0	174.0 ± 5.0	475.0 ± 75.0	212.3 ± 0.1	0.67	4186.3	115.6
Rajka (L209)	408.0 ± 21.8	20.3 ± 0.5	2.15 ± 0.13	10.9	919.0 ± 6	39.7 ± 0.2	27.1 ± 0.1	237.5 ± 3.5	247.5 ± 2.5	155.0 ± 5.0	173.9 ± 0.1	0.47	4068.6	57.5
Cultivar	TPC [mg/L]	ORAC [mmol/L]	TEAC [mmol/L]	Brix [°]	K^+ [mg/L]	Mg^{2+} [mg/L]	Ca^{2+} [mg/L]	Cu^{2+} [mg/L]	Mn^{2+} [mg/L]	Fe^{2+} [mg/L]	PO_4^{3-} [mg/L]	TA [% of malic]	Malic acid [mg/L]	Citric acid [mg/L]
Gewürzluiken (L211)	592.1 ± 43.8	16.1 ± 0.6	2.25 ±	10.5	715.5 ±	28.1 ±	23.0 ± 2.4	255.5 ±	165.0 ±	245.0 ±	176.2 ±	0.54	4009.8	57.5

			0.24		4.5	0.2		3.5	3.0	25.0	0.6			
Berlepsch (L214)	580.6 ± 9.4	16.9 ± 0.9	1.82 ± 0.30	12.0	872.0 ± 6	32.7 ± 0.1	33.6 ± 0.3	268.5 ± 8.5	164.0 ± 1.0	240.0 ± 10.0	171.6 ± 0.3	0.87	7362.8	28.4
Roter Herbstkalvill (L217)	875.9 ± 62.6	18.8 ± 2.3	4.11 ± 0.58	9.0	1016.0 ± 5	29.6 ± 0.0	21.9 ± 1.2	218.0 ± 2.0	127.5 ± 0.5	220.0 ± 20.0	187.3 ± 1.5	0.60	4951.0	115.6
Magna Super (L220)	223.3 ± 4.2	10.1 ± 1.2	0.94 ± 0.03	10.0	931.5 ± 1.5	39.7 ± 0.0	20.6 ± 0.3	372.0 ± 3.0	207.0 ± 3.0	230.0 ± 40.0	216.6 ± 0.4	0.60	5656.9	28.4
London Pepping (L224)	674.4 ± 42.6	23.7 ± 2.0	3.20 ± 0.32	10.0	1083.5 ± 1.5	27.2 ± 0.2	20.3 ± 0.8	178.0 ± 1.0	177.5 ± 2.5	325.0 ± 35.0	183.5 ± 1.4	0.67	6127.5	173.8
Ribston Pepping (L226)	182.3 ± 5.3	4.3 ± 1.7	0.85 ± 0.07	13.5	1572.5 ± 2.5	55.1 ± 0.2	20.6 ± 1.2	525.0 ± 3.0	276.0 ± 5.0	160.0 ± 10.0	420.3 ± 3.7	0.60	4127.5	57.5

n.d., not detectable; n.m., not measured; TPC, Total phenolic content; ORAC, Oxygen radical antioxidant capacity; TEAC, Trolox equivalent antioxidant capacity; TA, Titratable acidity.

Supplementary Table 1: Overview of the phytochemical composition of juice prepared from 88 apple cultivars collected from the region of Eferding/Upper Austria. Total phenolic content (TPC) was measured using the Folin-Ciocalteu method. Total antioxidant capacity was measured using TEAC and ORAC method. Quantitation of K^{+}, Mg^{2+} and Ca^{2+} was performed by ion chromatography; Cu^{2+}, Fe^{2+} and Mn^{2+} were quantitated by inductively coupled plasma mass spectrometry; phosphate (PO_4^{3-}) was determined using a phosphomolybdate method. Titratable acidity was determined by acid titration; malic and citric acid was quantified with HPLC. Error bars are based on the standard error of the mean (n = 3).

Supplementary Table 2: Study 1, 88 Organically grown Apple Cultivars.

	Dihydrochalcones		Flavan-3-ols					Flavonols						Hydroxycinnamic acids					
Cultivar	Phlor.	P-2-*O*-xylosyl-glucoside	EC	ECG	EGC	PCB 1	PCB 2	Q-3-*O*-rhamno.	Q-3-*O*-xylo.	Q-3-*O*-rutino.	Q-3-*O*-galacto.	Querc.	CA	Caff. acid	4-*p*-C-qui. acid	TPC (FC)	TPC (HPLC)	Anthocyanins
Rheinischer Krummstiel	3.9	9.8	14.5	8.7	n.d.	1.1	32.8	1.8	2.1	n.d.	0.6	3.4	335.9	1.2	1.2	975.4 ± 69.2	416.8	n.d.
Retina	0.4	0.6	n.d.	2.2	4.8	n.d.	4.4	n.d.	n.d.	n.d.	n.d.	n.d.	26.9	2.5	1.7	252.7 ± 18.8	43.5	n.d.
Maschanzker	14.2	23.1	13.8	14.6	9.7	5.1	51.7	0.6	3.4	+	1.8	3.8	247.6	5.4	29.7	1340.4 ± 66.4	424.6	n.d.
Carpetin (kleine Weinrenette)	4.7	11.2	33.8	12.0	n.d.	9.1	92.5	8.3	3.4	n.d.	0.6	5.8	156.3	3.7	9.0	903.1 ± 9.0	350.4	0.23
Sommerrambour	5.9	8.3	4.4	29.7	n.d.	n.d.	48.1	2.1	1.8	n.d.	n.d.	1.0	366.3	0.8	2.3	672.7 ± 24.9	470.5	n.d.
Dülmäner Rosenapfel	7.4	30.4	29.5	16.5	4.6	0.6	42.5	6.9	3.5	0.3	1.8	7.0	375.7	2.5	13.9	1008.7 ± 13.7	542.9	n.d.
Pilot	6.0	17.3	11.6	7.0	1.4	6.5	52.8	1.6	1.4	n.d.	+	1.0	322.1	7.0	26.5	1266.8 ± 26.2	462.3	n.d.
Roter Passamaner	11.4	13.6	104.7	46.2	38.2	20.7	170.0	25.2	41.1	15.7	26.4	49.2	441.8	10.1	19.3	819.2 ± 44.6	1033.7	n.d.
Schmidberger Renette	3.6	9.4	1.1	2.5	0.6	0.6	11.9	+	n.d.	n.d.	n.d.	n.d.	47.6	3.4	2.4	357.7 ± 16.5	83.3	n.d.
Gelber Edelapfel	7.6	19.7	7.3	27.5	6.9	3.1	66.4	2.1	1.3	+	n.d.	1.1	423.1	9.8	4.7	1279.0 ± 47.5	580.8	n.d.
Bismarck	4.9	11.8	4.0	51.0	n.d.	n.d.	42.8	1.1	1.6	0.5	n.d.	0.6	141.0	4.9	3.2	297.1 ± 5.9	267.3	n.d.
Schieblers Taubenapfel	0.8	4.4	1.8	6.4	3.1	1.7	16.4	0.6	0.3	n.d.	n.d.	0.3	76.9	3.1	5.2	578.0 ± 20.1	121.1	n.d.
Liberty	1.6	3.2	4.0	3.9	2.3	2.3	31.4	0.6	1.6	n.d.	0.3	1.3	170.6	4.4	3.3	634.2 ± 15.8	230.8	n.d.
Alkmene	2.6	5.1	4.4	31.1	n.d.	n.d.	30.3	1.3	1.4	n.d.	+	1.4	181.1	4.9	0.3	676.7 ± 19.0	264.1	n.d.
Jonathan	12.6	15.6	21.1	5.6	6.9	8.0	102.2	n.d.	n.d.	n.d.	n.d.	n.d.	776.5	16.2	24.5	1493.1 ± 46.7	989.2	n.d.
Herrenapfel	19.9	24.4	37.5	6.4	n.d.	n.d.	104.4	6.4	2.7	0.3	n.d.	3.7	484.0	8.2	16.2	1009.2 ± 70.4	714.2	n.d.
Spitzling	9.1	16.5	5.5	10.1	n.d.	4.0	n.d.	3.7	2.6	n.d.	0.8	2.9	257.1	4.5	36.7	939.4 ± 63.4	353.4	n.d.
Hausapfel	3.8	10.4	21.5	26.3	n.d.	11.6	28.1	1.4	0.5	n.d.	0.3	0.8	208.7	2.1	9.3	410.6 ± 1.8	324.8	n.d.
Spitzapfel	11.3	14.9	13.1	9.8	10.0	3.1	51.1	2.1	4.8	+	2.2	5.0	236.4	6.2	45.1	1092.8 ± 9.3	415.3	n.d.
Graue Herbstrenette	2.5	5.2	4.0	11.2	+	+	22.2	1.1	0.6	+	n.d.	0.3	131.0	5.0	2.0	624.1 ± 17.5	185.8	n.d.
Harberts Renette	23.6	21.4	55.3	5.3	3.5	10.8	192.2	2.4	2.6	+	n.d.	1.6	1209.2	11.3	17.5	2242.3 ± 75.8	1556.8	n.d.
Lesans Kalvill	1.9	6.0	5.8	3.1	3.1	1.4	25.6	1.4	1.1	+	+	1.0	141.7	3.8	9.7	819.7 ± 13.6	205.8	n.d.
Samareiner Rosmarien	5.4	14.5	14.9	6.2	n.d.	23.0	51.7	n.d.	n.d.	n.d.	n.d.	n.d.	92.5	3.9	14.9	846.2 ± 35.7	226.9	0.87
Berneder	3.4	6.7	8.0	5.3	n.d.	3.4	15.6	2.9	1.3	n.d.	+	1.6	168.7	3.5	5.4	296.3 ± 47.2	225.8	n.d.
Roter Boskoop	4.9	9.7	6.2	17.4	4.8	n.d.	51.1	4.8	2.2	0.3	n.d.	1.9	257.5	5.5	40.9	1135.6 ± 1.2	407.2	n.d.
Goldrenette Freiherr v. Berlepsch	11.8	15.2	12.7	10.6	n.d.	n.d.	41.4	2.2	0.8	n.d.	n.d.	1.1	362.8	8.5	16.9	524.2 ± 63.6	484.0	n.d.
Florianaer Rosmarin	5.0	4.1	6.5	8.7	2.7	3.4	28.1	1.6	3.5	n.d.	+	2.9	251.0	4.8	43.4	1049.5 ± 51.4	365.8	n.d.

Boikenapfel	5.9	14.8	2.9	12.9	n.d.	1.1	40.3	1.3	1.4	+	0.3	0.3	78.4	5.1	5.7	103.2 ± 1.1	170.6	n.d.
Roter Stettinger	11.6	40.9	19.6	11.2	n.d.	n.d.	117.5	1.1	2.9	n.d.	n.d.	0.6	781.8	9.4	37.8	1511.8 ± 99.1	1034.5	0.29
Kammerapfel	5.5	5.8	6.9	5.9	1.2	2.3	33.6	2.7	0.8	+	n.d.	1.3	179.1	6.0	7.3	800.3 ± 6.3	258.4	n.d.
Weißer Winter-Taffetapfel	6.6	19.6	51.3	27.7	7.1	17.3	77.5	3.2	3.2	n.d.	0.5	3.8	206.0	2.1	5.6	788.7 ± 55.1	431.6	0.23
Odenwälder	19.2	54.3	75.6	3.0.	n.d.	n.d.	338.1	1.0	3.4	n.d.	n.d.	1.0	1027.7	1.8	12.1	2275.6 ± 92.4	1564.0	0.39
Damason Renette	11.8	39.0	24.0	26.3	n.d.	n.d.	n.d.	3.8	2.6	+	+	1.3	736.6	n.d.	21.0	592.1 ± 23.9	866.7	n.d.
Champagner Renette	3.0	13.0	8.0	7.6	3.5	2.0	56.7	n.d.	n.d.	n.d.	n.d.	n.d.	323.5	8.1	51.3	1128.0 ± 35.8	476.5	n.d.
Hauxapfel	2.2	6.8	2.9	9.8	n.d.	n.d.	23.1	4.3	3.5	+	1.1	3.8	83.0	1.8	14.7	655.3 ± 46.9	157.1	n.d.
Glockenapfel	5.8	11.9	20.4	3.6	5.2	1.4	75.6	2.2	1.6	n.d.	0.5	1.0	425.0	7.3	8.9	1028.7 ± 45.2	570.3	n.d.
Zuccalmaglios Renette	14.3	16.9	43.3	10.4	2.1	3.1	37.5	5.4	7.5	+	1.6	5.0	284.5	5.7	12.7	2264.6 ± 21.0	450.2	n.d.
Roter James Grieve	2.8	7.0	16.0	10.6	4.6	n.d.	59.2	6.1	3.2	n.d.	n.d.	2.1	177.6	1.9	4.3	503.7 ± 7.5	295.3	n.d.
Weißer Passamaner	2.4	5.2	n.d.	16.2	n.d.	n.d.	42.5	3.7	2.7	+	0.3	2.6	136.1	32.5	0.5	401.7 ± 15.1	244.8	n.d.

	Dihydrochalcones		Flavan-3-ols					Flavonols					Hydroxycinnamic acids					
Cultivar	Phlor.	P-2-*O*-xylosyl-glucoside	EC	ECG	EGC	PCB 1	PCB 2	Q-3-*O*-rhamno.	Q-3-*O*-xylo.	Q-3-*O*-rutino.	Q-3-*O*-galacto.	Querc.	CA	Caff. acid	4-*p*-C-qui. acid	TPC (FC)	TPC (HPLC)	Anthocyanins
Plankenapfel	3.3	11.8	20.4	16.5	n.d.	n.d.	48.3	3.0	4.5	n.d.	+	9.4	82.3	4.1	4.1	671.2 ± 39.2	207.9	0.24
Riesenboikenapfel	2.2	8.8	4.4	6.7	2.5	3.1	26.4	2.4	4.6	+	1.0	3.8	203.9	5.8	0.5	828.5 ± 2.2	276.2	n.d.
Ananasrenette	3.0	13.3	10.5	6.7	0.8	2.3	77.8	1.8	1.3	+	+	1.4	350.2	4.4	30.7	708.8 ± 9.6	504.4	n.d.
Glasapfel	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	3.5	2.6	n.d.	0.3	6.9	n.d.	n.d.	n.d.	254.7 ± 28.1	13.3	0.10
Steirischer Maschanzker	7.3	15.5	7.3	9.5	n.d.	7.4	39.7	0.5	3.2	n.d.	n.d.	1.8	278.4	9.3	55.5	975.0 ± 18.7	435.4	n.d.
Pinova	1.8	1.9	0.7	3.1	n.d.	n.d.	10.0	1.0	1.6	n.d.	n.d.	0.6	135.7	3.5	7.0	428.6 ± 14.3	166.9	n.d.
Roter Griesapfel	11.9	16.2	10.9	7.0	n.d.	4.3	23.3	4.5	2.9	n.d.	1.8	6.7	266.8	5.9	27.2	1137.0 ± 38.9	389.3	n.d.
Gelber Bellefleur	2.6	8.4	5.1	4.2	n.d.	2.6	16.1	3.8	0.3	n.d.	n.d.	1.1	117.5	1.6	5.5	475.3 ± 9.9	168.8	n.d.
Rewena	1.5	2.6	2.9	1.4	n.d.	n.d.	7.5	n.d.	n.d.	n.d.	n.d.	n.d.	44.4	0.6	n.d.	221.0 ± 6.1	61.0	n.d.
Piros	+	n.d.	1.1	2.5	2.1	0.9	7.8	3.2	2.7	n.d.	0.3	2.7	26.0	1.4	0.1	274.9 ± 10.7	51.1	n.d.
Schöner v. Wiltshire	29.5	11.1	1.8	1.4	1.0	n.d.	13.6	13.4	18.4	0.3	9.3	25.2	98.1	4.3	6.3	610.4 ± 23.5	233.8	0.64
Prinzenapfel Fasslapfel	4.5	9.7	41.5	7.6	n.d.	10.2	58.1	n.d.	n.d.	n.d.	n.d.	n.d.	250.0	3.7	13.7	671.3 ± 77.9	398.9	n.d.
Kleiner Feiner	2.1	7.9	5.5	8.4	n.d.	3.7	21.4	2.2	1.1	n.d.	+	1.3	73.6	2.3	10.7	622.6 ± 5.0	140.3	n.d.
Geheimrat Oldenburg	19.0	32.7	13.1	18.2	n.d.	n.d.	114.4	8.6	8.9	n.d.	1.3	11.2	700.2	8.4	16.4	989.8 ± 24	952.6	n.d.
Deans Küchenapfel	7.2	4.2	16.0	6.4	1.9	12.5	43.1	5.4	9.7	+	2.7	7.8	200.3	6.9	20.1	950.2 ± 40.4	344.5	n.d.
Zabergau Renette	8.6	17.6	12.7	10.4	4.6	11.4	34.7	3.7	1.8	+	1.4	2.1	128.7	3.1	1.3	329.8 ± 30.2	242.2	n.d.
Kanada Renette	2.5	5.7	19.3	22.4	10.2	4.3	33.3	3.5	2.2	+	+	1.6	194.9	6.8	1.9	700.7 ± 4.5	308.9	n.d.
Winter-Goldparmäne	6.3	27.2	21.1	24.6	1.5	2.0	131.1	4.3	9.3	+	+	5.9	617.0	7.6	21.5	1869.9 ± 36	879.7	n.d.

	Dihydrochalcones		Flavan-3-ols					Flavonols					Hydroxycinnamic acids					
Cultivar	Phlor.	P-2-*O*-xylosyl-glucoside	EC	ECG	EGC	PCB 1	PCB 2	Q-3-*O*-rhamno.	Q-3-*O*-xylo.	Q-3-*O*-rutino.	Q-3-*O*-galacto.	Querc.	CA	Caff. acid	4-*p*-C-qui. acid	TPC (FC)	TPC (HPLC)	Anthocyanins
Grüner Boskoop	4.1	15.5	17.5	16.8	12.0	5.7	30.8	3.2	0.5	0.6	1.0	1.4	549.3	4.9	38.5	876.2 ± 37.9	701.7	n.d.
Relinda	1.7	10.9	3.6	8.1	n.d.	n.d.	21.7	n.d.	0.5	0.3	0.5	1.1	109.9	1.0	3.9	504.1 ± 13.6	163.2	n.d.
Pom. Kongreß	20.6	22.2	49.1	12.6	13.9	12.8	77.5	1.9	3.0	+	0.6	2.6	224.3	4.2	27.3	1315.0 ± 49.8	472.7	n.d.
Fasslapfel	11.8	45.9	41.8	41.5	n.d.	16.5	38.6	1.1	2.6	+	+	2.6	274.7	6.0	15.7	597.1 ± 37.2	499.0	n.d.
Royal Gala (F204)	+	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	n.d.	n.d.	n.d.	n.d.	n.d.	1.79	n.d.	0.3	185.8 ± 4.7	3.1	0.34
Winterzitrone (F208)	7.6	14.3	n.d.	6.1	n.d.	n.d.	10.0	1.9	2.4	+	1.6	7.5	65.0	n.d.	2.7	1029.6 ± 38.1	119.1	n.d.
Weißer Winterkalvill (F210)	5.0	4.9	2.8	2.2	n.d.	n.d.	0.8	2.0	1.2	+	+	1.4	75.3	n.d.	3.9	728.6 ± 43.0	99.6	n.d.
Stäubli 2 (F212)	4.9	13.7	n.d.	4.4	n.d.	n.d.	4.4	0.8	0.9	n.d.	0.4	2.2	42.9	n.d.	3.1	318.5 ± 18.1	77.7	n.d.
Christkindler (F213)	17.8	20.1	15.7	6.4	n.d.	12.0	55.0	0.4	1.1	n.d.	0.3	1.6	76.4	n.d.	44.4	1022.0 ± 3.5	251.2	0.70
Freyperg (F215)	1.3	1.6	n.d.	1.3	n.d.	n.d.	1.4	n.d.	n.d.	n.d.	n.d.	0.3	10.0	n.d.	1.6	370.3 ± 14.0	17.5	n.d.
Topaz (F216)	0.9	2.7	7.2	6.6	n.d.	1.8	0.5	n.d.	n.d.	+	n.d.	0.4	13.6	n.d.	n.d.	257.4 ± 10.6	33.7	n.d.
Florina (F218)	0.5	0.8	n.d.	n.d.	n.d.	n.d.	1.4	2.7	n.d.	n.d.	n.d.	0.8	6.3	n.d.	n.d.	224.2 ± 5.5	12.5	n.d.
Wachsrenette (F219)	1.4	2.9	4.4	n.d.	n.d.	n.d.	3.8	0.8	0.8	n.d.	0.3	3.3	40.8	n.d.	2.8	919.9 ± 21.1	61.3	n.d.
Discovery (F221)	3.7	9.9	7.2	3.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	139.4	1.3	3.9	836.8 ± 12.7	169.0	n.d.
Sponheimer Flurapfel (F222)	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	881.3 ± 4.2	n.d.	n.d.
Roter von Siemonffi (F223)	20.0	18.5	31.9	n.d.	n.d.	n.d.	34.1	1.1	1.1	n.d.	n.d.	1.6	275.6	2.4	5.7	1094.6 ± 30.2	392.0	0.35
Grüter Edelapfel (F225)	4.0	1.9	8.1	n.d.	n.d.	n.d.	3.5	3.3	1.1	0.3	0.3	0.8	41.4	n.d.	0.8	785.9 ± 14.3	65.2	0.30
Rheinischer Winterrambour (L200)	8.3	13.3	7.2	16.1	8.8	4.8	22.9	1.4	0.6	+	+	2.4	111.7	1.1	10.3	1087.4 ± 43.1	209.1	n.d.
Dr. Seeligs Orangenpepping (L201)	7.5	8.1	n.d.	21.1	n.d.	n.d.	14.4	0.3	n.d.	+	+	0.4	80.0	n.d.	5.8	527.8 ± 23.9	137.8	n.d.
Von Zuccalmaglios Renette (L202)	13.5	24.0	13.7	n.d.	n.d.	13.5	40.9	1.2	2.2	+	0.6	6.2	149.7	1.6	7.0	1035.7 ± 64.0	274.2	2.29
Mostzigeuner (L203)	10.3	14.4	n.d.	10.3	n.d.	n.d.	8.8	1.1	1.6	n.d.	n.d.	4.3	145.8	1.7	1.4	1283.5 ± 66.0	199.7	6.79
Blenheim (L205)	7.1	23.1	n.d.	13.6	3.3	3.9	8.5	+	0.4	+	n.d.	1.1	132.4	1.3	2.2	554.6 ± 6.5	197.1	n.d.
Hallauer Maienapfel (L206)	11.5	18.2	n.d.	17.2	22.1	18.4	33.2	1.4	2.4	+	0.4	3.0	62.8	n.d.	21.2	863.8 ± 20.4	211.9	0.74
Seeländer Reinette (L207)	2.3	3.7	3.6	6.9	n.d.	n.d.	5.0	0.3	0.4	+	n.d.	1.1	12.7	n.d.	1.7	270.9 ± 18.7	37.8	n.d.
Rajka (L209)	3.9	3.8	10.1	8.9	n.d.	n.d.	13.5	3.6	n.d.	n.d.	0.3	0.9	23.2	n.d.	4.7	408.0 ± 21.8	72.9	n.d.
Gewürzluiken (L211)	13.5	6.4	n.d.	35.9	21.0	6.3	27.6	0.8	1.9	n.d.	0.3	6.2	69.5	n.d.	17.0	592.1 ± 43.8	206.4	n.d.
Berlepsch (L214)	8.6	20.7	n.d.	16.4	n.d.	n.d.	11.1	n.d.	n.d.	+	n.d.	0.3	108.9	1.0	0.6	580.6 ± 9.4	167.7	n.d.
Roter Herbstkalvill (L217)	5.7	6.8	n.d.	7.2	28.8	3.6	12.0	0.3	0.3	n.d.	n.d.	1.1	72.0	n.d.	3.6	875.9 ± 62.6	141.4	0.23

Magna Super (L220)	1.3	2.7	n.d.	1.6	n.d.	n.d.	1.4	n.d.	n.d.	n.d.	n.d.	n.d.	10.1	n.d.	+	223.3 ± 4.2	17.2	n.d.
London Pepping (L224)	6.5	11.0	6.0	6.4	21.0	2.4	27.9	2.7	3.6	+	n.d.	3.6	67.7	n.d.	0.9	674.4 ± 42.6	159.8	n.d.
Ribston Pepping (L226)	2.8	8.2	n.d.	1.9	n.d.	n.d.	n.d.	n.d.	n.d.	+	n.d.	0.3	36.9	n.d.	3.3	182.3 ± 5.3	53.5	n.d.

n.d., not detectable; n.m., not measured; +, not quantifiable; PCB1, Procyanidin B1; EGC, Epigallocatechin; CA, Chlorogenic acid; PCB2, Procyanidin B2; Caff. acid, Caffeic acid; EC, Epicatechin; 4-*p*-C-qui. acid, 4-*p*-Coumarylquinic acid; ECG, Epicatechingallate; Phlor., Phloridzin; P-2-*O*-xylosyl-glucoside, Phloretin-2 ‘-*O*-xylosyl-glucoside; Q-3-*O*-rhamno., Quercetin-3-*O*-rhamnoside; Q-3-*O*-xylo., Quercetin-3-*O*-xyloside; Q-3-*O*-rutino., Quercetin-3-*O*-rutinoside; Q-3-*O*-galacto., Quercetin-3-*O*-galactoside; Querc., Quercetin; TPC, total phenolic content.

Supplementary Table 2: Overview of single polyphenol content of juice prepared from 88 apple cultivars collected from the region of Eferding/Upper Austria. Polyphenols were identified using HPLC-MS and were quantified with HPLC using known standards. Total anthocyanins were determined using a differential pH method. Error bars are based on the standard error of the mean (n = 3).

Supplementary Table 3: Study 2, 76 Organically grown Apple Cultivars. Tulln, Austria.

Cultivar	TPC [mg/L]	ORAC [mmol/L]	FRAP [mmol/L]	Brix [°]	K^+ [mg/L]	Mg^{2+} [mg/L]	Ca^{2+} [mg/L]	F^- [mg/L]	Cl^- [mg/L]	No^{3-} [mg/L]	Pentromenter	TA [% of malic]	Malic acid [mg/L]	Citric acid [mg/L]
Ananas Renette	788.50	24.83	8.84	14.0	3460.405	58.818	49.05	142.601	12.521	65.97	7.1	1.2	7.1	10303.90
Ananas Renette	382.60	19.82	7.30	11.7	2159.437	30.094	27.549	215.915	13.69	56.797	3.4	0.8	3.4	1656.90
Apfel aus Croncels	924.33	9.88	18.17	15.7	1051.672	17.002	12.305	136.088	6.155	18.278	8.2	0.8	8.2	1862.80
Bayrischer Brünnerling	476.38	8.13	9.03	13.1	3204.71	41.721	28.981	50.891	3.861	22.668	3.1	0.7	3.1	2468.60
Berlepsch	1716.00	23.99	18.28	14.0	1641.913	22.948	20.964	90.067	1.483	10.104	9.4	0.7	9.4	6009.80
Bohnapfel	890.25	23.38	24.30	12.0	1992.078	32.513	29.174	61.064	3	17.023	6.5	0.9	6.5	4068.60
Boikenapfel	591.08	20.00	15.76	13.5	1850.298	22.025	18.802	135.726	2.913	4.834	5.7	1.1	5.7	4615.70
Bramleys Seeding	565.96	16.07	13.94	13.0	2449.485	39.087	27.207	86.734	2.876	13.857	2.2	0.5	2.2	2303.90
Bratarsch I	442.75	9.00	12.68	13.9	2933.014	39.953	37.253	62.98	6.221	24.417	4.8	0.6	4.8	1892.20
Brauner Matapfel	731.95	7.14	22.62	14.0	1969.085	36.951	24.691	70.643	5.247	17.215	4.6	0.7	4.6	92.20
Breitarsch II	1231.42	22.47	12.14	13.9	1225.627	18.744	15.294	89.369	5.391	14.816	12.4	1.1	12.4	14892.20
Brünnerling	1064.50	19.16	8.25	12.3	1607.057	24.176	17.406	81.56	0	3.861	6.4	0.8	6.4	7774.50
Deans Küchenapfel	563.88	8.86	8.94	15.3	2252.241	26.262	26.137	74.493	0	14.185	5.7	0.7	5.7	1480.40
Edelrambour v. Winnitza	482.46	18.43	14.78	12.3	2100.26	29.581	29.578	120.075	3.725	13.954	8.2	0.8	8.2	5362.80
Enterprize	767.67	25.14	8.18	12.0	1484.956	31.294	42.093	75.255	4.072	12.492	4.6	0.7	4.6	8951.00
Florianer Rosmarin	406.88	17.98	16.46	11.9	1267.615	29.143	24.283	97.317	1.736	7.493	7.5	0.7	7.5	4656.90
Flovina	492.67	10.72	6.64	10.0	1451.827	21.429	10.42	54.611	0	15.481	4.1	0.7	4.1	6892.20
Geheimrat Dr. Oldenburg	718.04	8.81	10.03	14.3	1996.984	39.356	40.857	75.134	0	21.922	3.0	0.7	3.0	2539.20
Gewürzluiken	812.00	17.86	7.22	11.3	1832.899	26.882	16.718	201.709	0	26.111	4.4	0.5	4.4	68.60
Gloria Mundi	816.73	19.39	14.22	11.5	1066.791	25.243	25.605	226.494	2.429	7.856	5.9	0.5	5.9	3539.20
Goldparmäne	2984.50	28.73	24.24	14.7	911.787	26.065	23.671	70.407	3.15	9.426	6.9	1.1	6.9	9421.60
Goldrenette von Blenheim	604.33	6.81	11.09	12.0	1254.015	30.223	11.406	72.875	2.157	4.984	10.2	0.8	10.2	903.90
Goldrush	2050.13	31.31	20.14	14.2	1697.942	44.608	29.946	58.837	0	11.271	7.2	1.3	7.2	15774.50

Graue Renette	1128.04	34.58	10.23	12.0	1167.963	21.498	11.193	122.604	4.357	10.878	6.3	0.9	6.3	12245.10
Hausapfel	609.00	7.91	7.94	12.5	1125.604	21.329	12.286	81.692	3.376	12.006	5.7	0.8	5.7	2639.20
Hausmütterchen	748.50	15.85	11.63	13.1	935.09	19.534	11.178	74.788	0	13.112	11.0	0.9	11.0	98.10
Ilzer Rosenapfel	571.79	9.11	9.70	12.7	652.302	13.138	10.025	85.411	4.252	19.723	2.5	0.9	2.5	68.60
Ingol	1026.38	9.30	14.72	10.0	836.896	15.033	12.734	63.179	2.422	15.285	3.3	0.7	3.3	2745.10
Jakob Lebel	591.38	17.19	16.18	12.3	1019.718	16.625	17.196	80.103	7.202	23.834	5.9	0.9	5.9	9009.80
Jonathan	729.96	21.32	8.25	13.3	1757.002	29.817	14.286	133.197	0	11.768	9.3	1.1	9.3	9421.60
Kanada Renette	799.42	18.92	19.12	14.8	1317.454	30.223	21.283	59.676	2.801	12.831	5.7	0.8	5.7	5362.80
Kanada Renette	1712.63	12.01	27.11	12.4	1443.593	28.517	12.126	48.791	3.291	8.371	3.3	0.8	3.3	2751.00
Konstanzer	302.63	7.25	10.99	13.8	1586.545	30.504	16.127	59.634	4.154	11.96	6.9	0.7	6.9	209.80
Kronprinz Rudolf	642.67	23.33	7.96	11.1	1447.858	26.55	12.231	59.953	3.731	11.397	4.7	0.7	4.7	7951.00
Lansberger Renette	565.67	18.18	12.12	10.9	1157.655	22.523	8.269	34.999	2.367	8.693	9.5	0.5	9.5	2774.50
Lavanttaler Bananenapfel	1231.92	23.93	30.88	14.0	1242.826	19.027	7.487	49.048	3.851	11.804	5.9	0.9	5.9	6833.40
Lederrenette	611.79	7.91	9.36	13.0	989.209	18.176	11.053	107.518	1.7	7.133	3.1	0.5	3.1	127.50
Liberty	173.46	7.17	7.52	12.2	1847.808	36.34	17.38	56.687	3.158	10.265	5.3	0.7	5.3	498.10
Luna	776.52	20.43	8.03	13.3	1191.192	20.542	7.701	41.674	3.606	10.256	13.0	0.7	13.0	5598.10
Maschanzker	788.50	24.83	8.84	12.0	1362.572	23.154	9.9	56.109	4.163	10.837	7.9	0.7	7.9	4480.40
Maschanzker	1157.08	23.09	18.00	16.0	1408.632	33.313	0	56.045	2.113	7.993	4.0	0.5	4.0	2921.60
Maschschankzka	215.54	5.02	19.66	11.5	1084.574	23.808	11.382	30.313	2.312	9.044	5.0	0.9	5.0	421.60
Maunzenapfel	1544.71	8.18	17.82	12.0	1096.864	28.153	27.382	65.109	3.834	7.186	5.3	0.7	5.3	992.20
Minister v. Hammerstein	235.54	7.83	8.86	10.9	2154.4	46.23	19.207	58.812	3.277	12.408	4.6	0.7	4.6	4303.90
Olderling	1562.42	12.15	9.86	14.0	1806.505	61.634	28.357	30.419	2.942	16.41	6.6	0.8	6.6	4398.10
Ontario	262.33	6.11	8.02	13.9	1457.808	29.326	16.237	34.876	3.267	13.409	6.1	0.9	6.1	6656.90
Pilot	1410.33	44.17	9.42	13.0	1571.765	34.25	25.563	80.32	4.42	31.319	8.8	0.8	8.8	3068.60
Plankenapfel	784.00	24.04	17.02	12.0	1561.726	42.106	36.353	52.08	3.584	16.754	8.8	1.1	8.8	9656.90
Reanda	999.75	42.63	10.38	14.9	871.162	23.86	15.487	34.458	2.599	12.802	-0.03	0.7	4.9	2609.80

Rebella	402.43	7.99	18.81	13.0	1520.542	42.774	31.009	38.621	2.512	16.887	4.9	0.6	3.3	2980.40
Remo	603.88	7.99	8.61	12.9	1394.812	38.477	27.485	61.895	3.647	9.772	3.3	0.7	4.3	186.30
Retina	222.43	7.40	19.76	12.0	1840.312	39.023	25.292	62.645	3.018	8.677	4.3	0.5	8.3	892.20
Rewena	310.67	17.15	12.96	12.0	1840.312	39.023	25.292	52.761	3.9	0	8.3	0.8	6.1	4639.20
Rewena	289.83	18.71	12.68	12.5	1309.737	43.956	21.611	59.897	0	7.977	6.1	0.5	3.2	1251.00
Rheinischer Krummstiel	721.38	9.61	15.39	13.0	1213.309	26.965	23.004	89.274	2.691	11.843	3.2	0.8	6.2	2245.10
Rohling	1072.68	21.94	18.84	14.0	1931.438	62.436	0	48.172	2.667	9.23	6.2	1.1	10.1	68.60
Roter Boskoop	1593.04	40.53	17.49	15.0	1636.506	34.377	29.83	132.416	16.616	12.173	10.1	1.3	8.8	15127.50
Roter Herbstkalvill	995.96	20.19	9.86	11.9	1502.336	28.58	15.434	40.835	31.588	11.708	8.8	0.8	4.8	7715.70
Roter Krickapfel	1382.00	33.38	10.45	16.5	1357.618	40.837	28.084	43.953	1.774	7.906	4.8	1.2	13.9	10786.30
Roter Passamaner	1114.83	26.87	62.39	12.9	1417.655	33.703	15.331	74.23	2.55	7.524	13.9	0.5	7.3	4892.20
Roter Settiner	1354.58	20.48	20.10	14.0	1508.959	44.828	15.059	81.619	2.034	6.935	7.3	0.7	7.2	7539.20
Roter Winterrambour	593.50	7.20	8.25	10.9	811.941	21.399	11.703	74.641	1.913	8.941	7.2	0.5	5.8	4127.50
Rubiner	1114.83	21.39	19.26	12.9	835.807	20.572	12.472	45.984	1.921	9.414	5.8	0.5	6.5	127.50
Schmidberger Renette	606.24	7.99	38.17	13.0	1882.246	41.73	18.752	69.03	0.938	16.232	6.5	1.2	8.1	11892.20
Schöner von Boskoop	288.46	10.28	7.81	10.0	1029.521	18.564	12.533	50.639	0	10.473	8.1	1.3	8.2	
Schweizer Glockenapfel	1139.92	21.39	11.33	12.0	1022.81	16.089	16.952	72.053	0	10.822	8.2	0.9	6.2	3803.90
Seidenbrünnerling	187.21	6.51	8.86	14.5	1672.762	30.5	14.309	37.952	1.845	10.18	6.2	0.8	6.7	68.60
Selena	1030.71	20.95	14.36	10.9	1493.983	26.307	20.287	65.575	4.989	15.2	6.7	0.5	4.6	3362.80
Siebenkant	268.17	24.30	7.61	10.0	1275.792	27.661	14.315	38.262	3.081	8.978	4.6	0.9	12.5	7892.20
Spät blühender Taffelapfel	540.00	22.39	16.18	12.5	1622.844	36.318	21.041	48.63	2.859	8.514	12.5	0.9	4.2	3715.70
St. Pauler Weinapfel	1795.79	22.04	34.80	8.0	1121.471	20.708	13.778	58.898	6.018	17.797	4.2	1.7	10.9	22421.60
Taubenapfel	3254.75	100.25	27.62	10.0	1306.134	26.805	13.842	38.281	3.519	12.755	10.9	0.5	3.8	2621.60
Taubenapfel Gurten	539.83	8.32	8.61	11.0	1310.733	30.738	14.115	45.023	4.343	18.254	3.8	1.1	6.3	3986.30
Weißer Grießapfel	565.25	7.45	8.53	9.9	1741.793	52.393	26.73	138.406	43.238	22.954	6.3	1.1	7.8	5598.10
Wiltshire	1528.17	24.33	22.48	12.0	1939.053	35.635	24.238	70.626	4.314	10.998	7.8	0.7	8.1	2251.00

Zigeuner Apfel	513.46	6.11	9.03	11.0	1843.857	47.402	25.254	36.797	1.861	10.974	8.1	0.7	5.9	498.10
Cultivar	**TPC [mg/L]**	**ORAC [mmol/L]**	**TEAC [mmol/L]**	**Brix [°]**	**K^+ [mg/L]**	**Mg^{2+} [mg/L]**	**Ca^{2+} [mg/L]**	**Cu^{2+} [mg/L]**	**Mn^{2+} [mg/L]**	**Fe^{2+} [mg/L]**	**Pentrometer**	**TA [% of malic]**	**Malic acid [mg/L]**	**Citric acid [mg/L]**

n.d., not detectable; n.m., not measured; TPC, Total phenolic content; ORAC, Oxygen radical antioxidant capacity; TEAC, Trolox equivalent antioxidant capacity; TA, Titratable acidity.

Supplementary Table 4: Study 2, 76 Organically grown Apple Cultivars. Tulln, Austria.

	Dihydrochalcones		Flavan-3-ols					Flavonols					Hydroxycinnamic acids		
Cultivar	**Phlor.**	**P-2-*O*-xylosyl-glucoside**	**Epi**	**ECG**	**EGC**	**PCB1**	**PCB2**	**Q-3-*O*-rhamno.**	**Q-3-*O*-xylo.**	**Q-3-*O*-rutino.**	**Q-3-*O*-galacto.**	**Querc.**	**CGA**	**Caff. acid**	**CQA**
Ananas Renette	18.5	53.4	98.9	36.4	1.2	13.4	151.7	1.92	4.31	1.44	4.63	5.11	144.2	3.2	2.5
Apfel aus Croncels	24.0	47.6	20.4	22.7	2.5	12.2	60.3	2.88	4.79	1.28	6.87	5.91	368.0	4.5	4.4
Bayrischer Brünnerling	8.5	7.4	12.4	7.6	4.8	1.4	54.4	0.16	0.80	0.16	2.24	1.76	264.1	6.2	9.8
Berlepsch	3.6	5.4	5.1	5.9	1.9	0.9	8.9	0.32	2.08	0.16	2.56	1.60	97.3	2.0	2.3
Bohnapfel	10.4	17.0	52.7	16.0	5.8	8.0	51.4	0.64	3.19	0.32	3.35	3.51	173.1	0.4	11.5
Boikenapfel	15.5	48.1	1.1	22.7	3.5	2.6	22.8	0.16	1.92	0.00	2.40	2.08	495.2	9.0	11.9
Bramleys Seeding	7.0	8.1	31.3	9.2	3.7	14.8	77.5	0.96	4.63	0.16	3.83	2.24	281.5	1.5	9.5
Bratarsch I	20.7	13.1	128.0	15.1	2.7	6.5	48.9	2.24	7.51	1.28	6.23	5.11	233.2	7.8	60.5
Brauner Matapfel	4.4	7.5	5.8	7.3	2.1	2.6	11.7	1.60	3.35	0.16	2.24	1.92	244.2	2.2	9.6
Breitarsch II	11.3	16.0	6.5	10.1	1.0	2.0	9.4	1.12	2.08	0.64	2.40	2.24	98.3	3.4	4.4
Brünnerling	1.8	2.7	8.7	8.7	2.7	3.1	7.5	0.32	0.96	0.00	0.48	0.96	89.6	3.8	1.7
Deans Küchenapfel	5.9	7.4	5.5	14.3	5.0	3.4	19.2	0.80	3.35	0.48	3.67	4.15	385.9	6.9	6.2
Edelrambour v. Winnitza	6.8	7.4	11.3	10.6	2.7	3.7	8.6	0.48	3.83	0.16	4.15	2.24	185.7	3.8	4.3
Enterprize	4.8	7.4	9.5	5.3	0.6	4.8	23.6	0.64	2.24	0.96	5.59	2.56	159.5	2.1	4.2
Florianer Rosmarin	3.3	4.1	7.3	9.0	1.5	2.3	16.4	0.48	4.79	0.00	6.39	3.19	169.4	2.8	12.2
Flovina	4.6	12.4	25.8	6.7	10.6	14.2	46.4	0.48	2.24	0.00	2.08	1.12	251.5	3.9	7.0
Geheimrat Dr. Oldenburg	12.1	8.1	6.9	4.2	1.2	2.0	14.2	1.12	2.72	0.32	0.64	0.16	208.7	3.6	10.1
Gewürzluiken	5.0	7.7	62.5	14.3	18.9	58.8	94.2	0.64	3.51	0.32	3.04	2.72	408.0	3.9	12.3
Gloria Mundi	1.8	3.2	2.2	6.7	2.5	2.8	8.9	0.16	1.92	0.00	0.96	0.80	55.3	1.2	2.8
Goldparmäne	1.5	1.3	1.5	6.7	2.3	4.3	4.4	0.32	0.80	0.32	0.80	0.64	22.4	1.7	5.0
Goldrenette von Blenheim	1.5	1.5	0.4	3.4	1.4	1.4	2.2	0.16	0.64	0.00	0.48	0.16	15.1	0.5	3.1
Goldrush	0.6	0.8	3.6	4.2	3.5	2.8	0.6	0.96	0.96	0.00	0.80	0.32	9.7	1.3	1.1

Graue Renette	11.1	17.5	47.6	8.7	11.8	22.7	90.3	0.80	2.88	0.64	2.88	2.08	411.7	4.7	33.1
Hausapfel	0.7	1.8	0.7	3.4	1.4	9.7	5.0	0.32	1.12	0.16	1.28	0.80	32.5	0.3	1.0
Hausmütterchen	6.5	28.3	16.7	7.3	3.7	12.8	39.7	1.28	4.63	0.48	3.83	2.40	312.8	2.9	4.9
Ilzer Rosenapfel	4.0	15.6	2.9	17.4	1.0	4.0	10.3	0.48	2.24	0.16	1.92	0.80	150.9	2.8	3.7
Ingol	6.3	25.4	2.9	9.5	1.7	7.1	24.2	0.64	2.40	0.16	3.19	3.35	110.9	1.6	7.0
Jakob Lebel	2.8	5.3	1.1	2.2	3.5	2.8	8.3	0.32	2.08	0.48	2.24	0.96	163.4	0.0	0.8
Jonathan	2.4	4.2	3.3	1.1	1.5	4.5	12.2	0.32	1.28	0.32	1.60	0.64	141.5	1.4	7.1
Kanada Renette	4.0	6.6	19.6	19.0	2.3	6.5	70.6	2.24	0.64	0.64	2.24	1.92	222.8	2.7	15.4
Kanada Renette	0.8	2.3	4.7	5.0	1.9	4.0	5.3	0.32	2.56	0.16	2.56	0.64	19.6	0.5	1.1
Konstanzer	0.3	0.2	5.5	2.5	1.9	6.8	1.9	0.48	0.96	0.00	0.32	0.32	1.5	0.0	0.8
Kronprinz Rudolf	0.3	7.3	5.5	2.5	0.0	7.1	5.6	0.80	1.60	0.16	0.64	2.72	8.5	1.1	2.3
Lansberger Renette	1.2	1.4	1.1	2.0	3.3	1.4	6.4	0.32	3.99	0.96	4.31	3.51	55.4	2.6	3.2
Lavanttaler Bananenapfel	0.5	2.5	3.6	1.4	1.0	0.6	6.9	0.32	0.64	0.32	0.64	0.64	62.1	0.9	1.7
Lederrenette	2.0	1.5	6.5	2.8	2.1	2.6	22.5	0.64	0.96	0.00	0.96	0.48	15.3	1.1	0.3
Liberty	6.6	22.3	5.8	15.4	1.5	1.1	18.1	0.96	2.72	0.16	2.56	1.92	133.7	3.7	2.0
Luna	3.8	4.9	0.4	4.5	2.9	1.4	4.2	0.32	0.96	0.16	0.80	0.32	61.6	0.9	5.8
Maschanzker	6.6	8.9	12.0	4.5	2.1	1.7	27.2	0.64	2.24	0.16	2.08	1.60	386.9	4.7	4.4
Maschanzker	1.7	3.8	6.2	1.7	1.5	2.6	28.1	0.48	0.96	0.16	0.96	0.96	180.6	1.4	7.4
Maschschankzka	2.4	3.5	12.4	3.4	0.6	3.4	18.1	0.48	1.44	0.00	1.12	1.76	104.8	0.7	0.7
Maunzenapfel	1.1	0.9	4.4	6.4	6.6	0.6	3.9	0.16	0.64	0.00	0.64	0.48	47.6	1.0	0.8
Minister v. Hammerstein	0.1	0.1	0.7	0.8	1.4	2.6	1.9	0.32	0.16	0.00	0.16	0.32	1.7	0.0	0.4
Oldenwälder	7.0	26.6	19.3	26.1	1.9	3.1	79.4	0.96	1.60	0.32	2.40	1.44	212.6	1.6	3.1
Olderling	1.1	1.6	0.7	1.7	1.4	0.6	3.9	0.16	0.32	0.16	0.96	0.32	21.9	0.3	1.3
Ontario	0.5	1.3	1.1	1.1	1.2	0.6	1.7	0.16	0.64	0.00	0.64	0.48	24.7	0.5	1.1
Pilot	5.9	6.0	1.8	4.8	0.6	0.9	17.5	0.00	0.64	0.00	0.32	0.48	166.3	0.5	0.4
Plankenapfel	0.4	0.3	0.4	0.6	0.4	0.0	0.6	0.00	0.16	0.00	0.16	0.00	6.6	0.1	0.0
Reanda	0.1	0.4	0.0	0.0	0.0	0.3	0.8	0.00	0.00	0.00	0.00	0.00	1.5	0.0	0.0
Rebella	2.1	4.7	1.1	11.8	1.9	3.7	2.8	0.48	1.76	0.16	1.76	0.80	22.6	0.9	0.4
Remo	2.7	4.4	1.5	7.6	5.0	2.8	4.4	0.32	1.92	0.48	4.63	0.96	50.7	3.2	1.6

	Dihydrochalcones		Flavan-3-ols					Flavonols					Hydroxycinnamic acids		
Cultivar	**Phlor.**	**P-2-*O*-xylosyl-glucoside**	**EC**	**ECG**	**EGC**	**PCB1**	**PCB2**	**Q-3-*O*-rhamno.**	**Q-3-*O*-xylo.**	**Q-3-*O*-rutino.**	**Q-3-*O*-galacto.**	**Querc.**	**CA**	**Caff. acid**	**4-*p*-C-qui. acid**
Retina	0.1	0.2	0.0	0.3	0.0	0.0	0.0	0.00	0.00	0.00	0.00	0.00	1.5	0.1	0.1
Rewena	0.1	0.2	0.0	0.6	0.0	0.6	0.6	0.00	0.00	0.00	0.00	0.00	1.7	0.2	0.2
Rewena	0.0	0.3	0.0	0.6	0.0	0.3	0.3	0.00	0.00	0.00	0.00	0.00	2.0	0.0	0.0
Rheinischer Krummstiel															0.0

n.d., not detectable; n.m., not measured; +, not quantifiable; PCB1, Procyanidin B1; EGC, Epigallocatechin; CA, Chlorogenic acid; PCB2, Procyanidin B2; Caff. acid, Caffeic acid; EC, Epicatechin; 4-*p*-C-qui. acid, 4-*p*-Coumarylquinic acid; ECG, Epicatechingallate; Phlor., Phloridzin; P-2-*O*-xylosyl-glucoside, Phloretin-2‘-*O*-xylosyl-glucoside; Q-3-*O*-rhamno., Quercetin-3-*O*-rhamnoside; Q-3-*O*-xylo., Quercetin-3-*O*-xyloside; Q-3-*O*-rutino., Quercetin-3-*O*-rutinoside; Q-3-*O*-galacto., Quercetin-3-*O*-galactoside; Querc., Quercetin; TPC, total phenolic content.

Supplementary Table 5: Study 2, comparison of averaged Phase 1 & Phase 2 values.

Cultivar	Brix		TA		TPC	
	Phase 1	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*
Roter Boskoop	14.40	15.73	1.34	2.46	995.96	3375.15
Schmidberger Renette	12.07	12.67	1.21	1.23	288.46	514.63
Oldenwälder	12.00	11.03	0.67	1.23	1562.42	3359.83
Deans Küchenapfel	12.33	12.50	0.81	1.23	1064.50	3346.86
Kanada Renette	13.33	14.87	1.07	1.45	729.96	1658.21
Rheinischer Krummstiel	11.00	10.43	0.81	1.01	1072.68	2147.25
Rewena	13.83	14.33	0.81	0.78	289.83	635.25
Berlepsch	13.07	16.00	0.67	1.01	476.38	501.22
Maschanzker	13.40	12.13	0.54	0.78	215.54	1930.42
Liberty	13.00	13.83	0.54	0.67	611.79	478.70

Phase 1 is defined as the averaged values, taken in triplicates, of pure juice contaning more than one apple of the same cultivar.

Phase 2 is defined as the avereaged values of three like apple cultivars, with measurements taken in triplicate.

Supplementary Table 6: Study 2, comparison of averaged Phase 1 & Phase 2 values.

Cultivar	ORAC		FRAP		Maleic acid		Citric acid	
	Phase 1	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*
Roter Boskoop	20.19	34.41	9.86	48.70	15127.47	12249.04	290.05	193.15
Schmidberger Renette	10.28	20.29	7.81	26.58	11892.18	12145.12	231.91	270.67
Oldenwälder	12.15	23.65	9.86	52.14	4303.94	3029.43	34.23	28.42
Deans Küchenapfel	19.16	17.35	8.25	43.83	7774.53	8303.94	80.74	274.54
Kanada Renette	21.32	16.84	8.25	38.22	9421.59	7009.82	115.63	57.49
Rheinischer Krummstiel	21.94	18.38	18.84	35.33	2245.12	1872.57	28.42	28.64
Rewena	18.71	10.60	12.68	11.29	4639.24	3015.71	45.86	20.67
Berlepsch	8.13	6.38	9.10	10.43	2468.65	2205.90	10.98	5.16
Maschanzker	5.02	16.67	17.25	15.70	2921.59	2625.51	34.23	105.94
Liberty	7.91	11.66	9.28	13.58	127.47	366.69	10.98	5.16

Phase 1 is defined as the averaged values, taken in triplicates, of pure juice contaning more than one apple of the same cultivar.

Phase 2 is defined as the avereaged values of three like apple cultivars, with measurements taken in triplicate.

Supplementary Table 7: Study 2, comparison of averaged Phase 1 & Phase 2 values.

Cultivar	**K+**		**Mg2+**		**Ca^{2+}**		**CL-**	
	Phase 1	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*
Roter Boskoop	3204.71	2517.95	41.72	66.32	28.98	73.78	3.86	13.36
Schmidberger Renette	1641.91	1728.98	22.95	23.62	20.96	22.68	1.48	9.53
Oldenwälder	1451.83	1519.59	21.43	21.25	10.42	19.12	0.00	7.34
Deans Küchenapfel	1066.79	1649.55	25.24	21.82	25.61	41.88	2.43	75.57
Kanada Renette	1254.02	2145.42	30.22	39.39	11.41	23.34	2.16	7.15
Rheinischer Krummstiel	836.90	1427.85	15.03	17.50	12.73	23.11	2.42	6.30
Rewena	1561.73	2067.75	42.11	25.40	36.35	19.45	3.58	7.14
Berlepsch	1882.25	1955.19	41.73	28.04	18.75	24.51	0.94	7.29
Maschanzker	1022.81	1837.59	16.09	22.45	16.95	36.72	0.00	10.44
Liberty	1275.79	1138.68	27.66	11.21	14.32	10.25	3.08	5.01

Phase 1 is defined as the averaged values, taken in triplicates, of pure juice contaning more than one apple of the same cultivar.

Phase 2 is defined as the avereaged values of three like apple cultivars, with measurements taken in triplicate.

Supplementary Table 8: Study 2, comparison of averaged Phase 1 & Phase 2 values.

Cultivar	**Phloz**		**Phloz-2-O**		**Epi**		**ECG**	
	Phase 1	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*
Roter Boskoop	8.48	7.33	7.35	7.12	12.36	56.97	7.56	2.52
Schmidberger Renette	3.62	4.83	5.40	2.73	5.09	25.94	5.88	5.04
Oldenwälder	4.61	16.90	12.40	6.48	25.82	84.36	6.72	3.27
Deans Küchenapfel	1.78	8.77	3.15	3.62	2.18	63.88	6.72	1.49
Kanada Renette	1.54	12.62	1.50	8.71	0.36	61.58	3.36	0.56
Rheinischer Krummstiel	2.83	6.28	5.30	3.24	1.09	74.55	2.24	1.77
Rewena	0.40	4.68	0.30	3.37	0.36	21.33	0.56	0.75
Berlepsch	0.45	5.32	1.05	2.46	0.36	9.82	0.56	5.04
Maschanzker	5.26	14.65	5.70	14.41	11.64	29.94	7.28	4.11
Liberty	0.00	3.23	0.00	2.28	0.00	33.94	0.00	53.61

Phase 1 is defined as the averaged values, taken in triplicates, of pure juice contaning more than one apple of the same cultivar.

Phase 2 is defined as the avereaged values of three like apple cultivars, with measurements taken in triplicate.

Supplementary Table 5: Study 2, comparison of averaged Phase 1 & Phase 2 values.

Cultivar	**EGC**		**PCB1**		**PCB2**		**QU-3-O-rh**		**QU-3-O-x**	
	Phase 1	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*
Roter Boskoop	4.83	1.48	1.42	16.38	54.44	118.33	0.16	0.69	0.80	1.06
Schmidberger Renette	1.93	2.45	0.85	7.39	8.89	35.37	0.32	0.27	2.08	0.43
Oldenwälder	10.62	6.05	14.20	35.98	46.39	160.00	0.48	1.01	2.24	2.45
Deans Küchenapfel	2.51	1.67	2.84	12.50	8.89	130.28	0.16	0.96	1.92	2.50
Kanada Renette	1.35	2.32	1.42	8.81	2.22	91.39	0.16	1.22	0.64	1.86
Rheinischer Krummstiel	3.47	1.03	2.84	8.24	8.33	130.00	0.32	0.69	2.08	1.06
Rewena	0.39	6.18	0.00	5.30	0.56	36.57	0.00	2.18	0.16	9.74
Berlepsch	0.00	1.54	0.57	5.68	0.56	29.54	0.00	0.21	0.00	0.75
Maschanzker	2.12	2.83	5.40	6.06	24.17	74.44	0.00	1.97	0.00	4.42
Liberty	0.00	2.12	0.00	9.56	0.00	53.61	0.00	1.54	0.00	3.73

Phase 1 is defined as the averaged values, taken in triplicates, of pure juice contaning more than one apple of the same cultivar.

Phase 2 is defined as the avereaged values of three like apple cultivars, with measurements taken in triplicate.

Supplementary Table 5: Study 2, comparison of averaged Phase 1 & Phase 2 values.

Cultivar	**QU-3-O-g**		**QU**		**CGA**		**CAFF**		**CQA**	
	Phase 1	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*	*Phase 1*	*Phase 2*
Roter Boskoop	2.24	0.27	1.76	0.43	264.12	330.16	6.22	0.00	9.80	10.30
Schmidberger Renette	2.56	0.21	1.60	0.43	97.28	144.22	2.00	2.53	2.26	2.55
Oldenwälder	2.08	1.97	1.12	1.86	251.53	343.25	3.90	7.65	7.04	9.06
Deans Küchenapfel	0.96	1.49	0.80	1.86	55.27	344.27	1.19	3.75	2.76	3.37
Kanada Renette	0.48	0.69	0.16	1.12	15.14	315.99	0.54	9.45	3.12	3.75
Rheinischer Krummstiel	2.24	0.37	0.96	0.69	163.44	386.85	0.00	9.34	0.75	1.26
Rewena	0.16	1.60	0.00	1.60	6.63	115.65	0.11	4.56	0.00	0.42
Berlepsch	0.00	0.69	0.00	0.91	10.37	145.18	0.22	5.34	0.15	0.47
Maschanzker	0.00	4.15	0.00	3.25	248.47	483.67	1.73	11.62	9.40	24.57
Liberty	0.00	7.95	0.00	1.33	0.00	274.55	0.00	7.95	0.00	9.88

Phase 1 is defined as the averaged values, taken in triplicates, of pure juice contaning more than one apple of the same cultivar.

Phase 2 is defined as the avereaged values of three like apple cultivars, with measurements taken in triplicate.