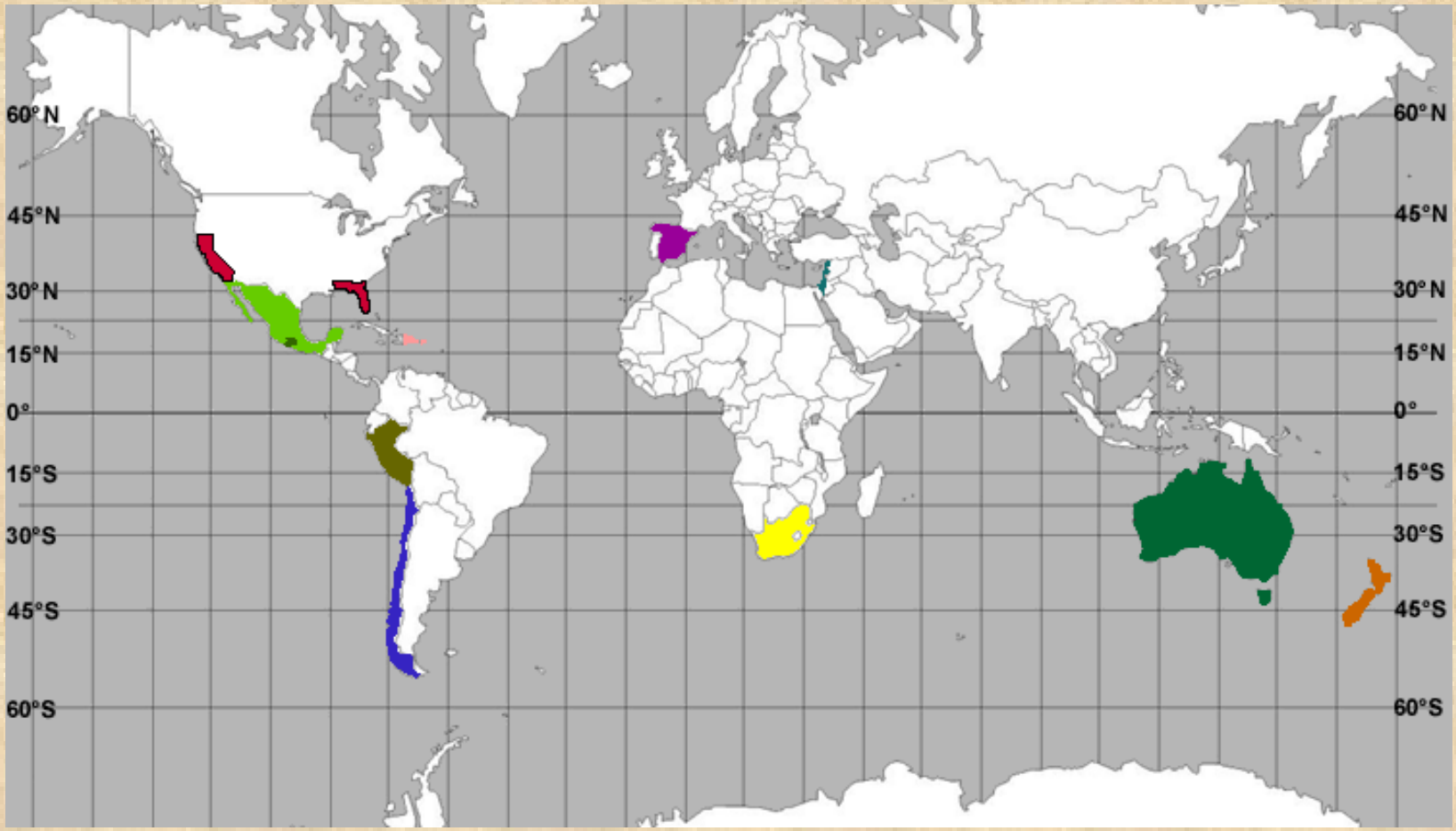
Evaluation of Phenolic Content in Avocado Fruit and Its By-Products

A.M Gómez-Caravaca, A. López-Cobo, V. Verardo, F. Pasini, M.F. Caboni, A. Segura-Carretero, A. Fernández-Gutiérrez

Department of Analytical Chemistry, University of Granada, Spain
Research and Development of Functional Food Centre (CIDAF), Granada, Spain
Department of Chemistry and Physic, University of Almeria, Spain
Inter-departmental Center of Industrial Agri-Food Research, University of Bologna, Italy
Department of Agro Food Science and Technology, University of Bologna, Italy

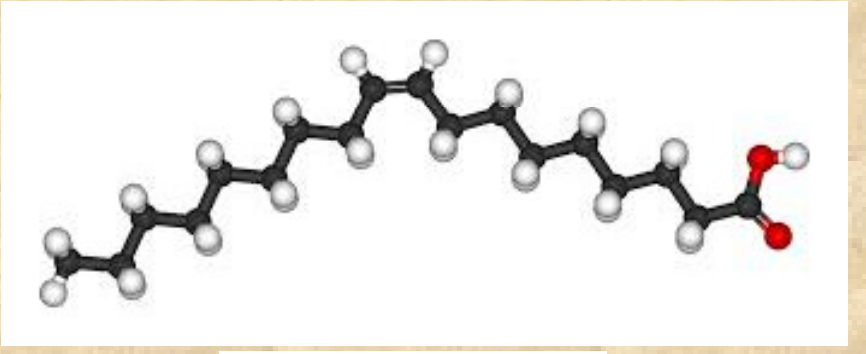
5th Euro-Global Summit and Expo on Food & Beverages

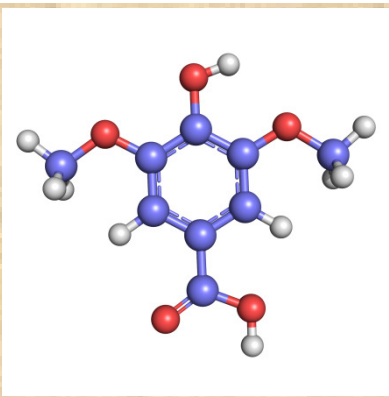
Avocado (*Persea americana* Mill.)



Avocado Composition

- ✓ High amount of oil (15-20 % of the pulp weight) mainly composed of unsaturated fatty acids, predominantly oleic acid.
- ✓ Fibre
- ✓ Vitamins B and E
- ✓ Ascorbic acid
- ✓ Carotenoids
- ✓ Sterols
- ✓ Phenolic compounds



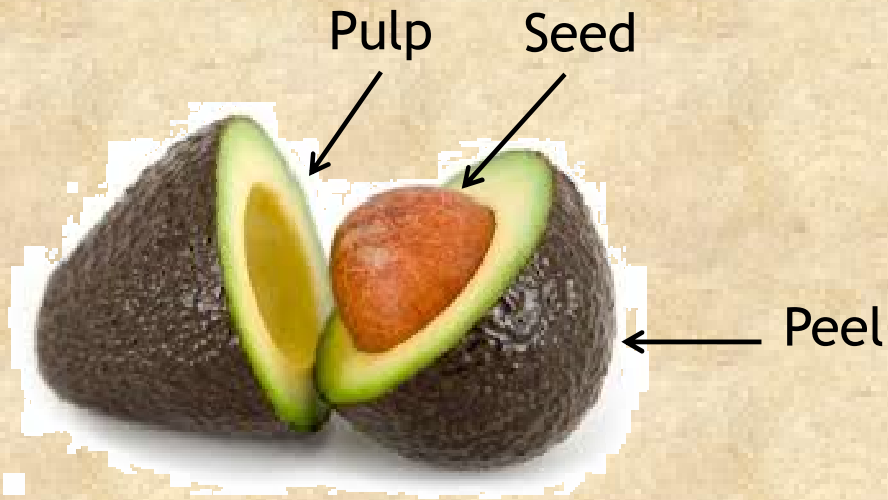


Avocado Consumption

Phenolic compounds health benefits

The polyphenol fraction of food has been widely studied in the last years because of the healthy benefits that these compounds have proven to provide. Its consumption has been linked to the decrease of the risk of diseases associated to oxidative stress, such as cancer and cardiovascular diseases

Avocado and its by-products



Large consume of avocado

↓

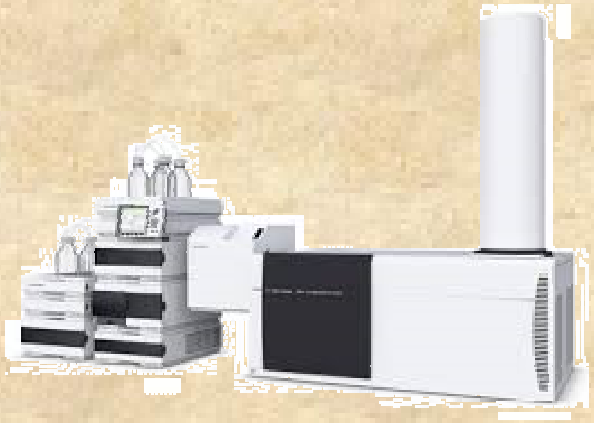
High production of avocado by-products

↓

Higher antioxidant activities than avocado pulp → Potential source of natural antioxidants to be used as nutraceuticals and food ingredients

Aim of the study

- ✓ Determine phenolic compounds in avocado pulp and its by-products (seed and peel) by using HPLC-DAD-qTOF-MS.
- ✓ Determine oligomeric proanthocyanidins specifically by HPLC-FLD
- ✓ Antioxidant activity by ABTS, FRAP and ORAC



Experimental section

Seed

Hass Avocado

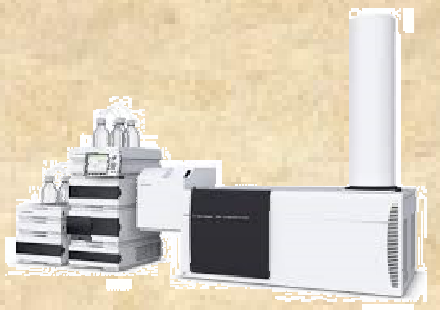
Peel

Pulp

- Lyophilization
- Extraction
- Analytical separation
- Identification and quantification

Analyses performed

Phenolic compounds → Solid-Liquid extraction $MeOH/H_2O$ 80/20 →



HPLC-DAD-QTOF-MS

Oligomer proanthocyanidins → Solid-Liquid extraction Acetone/H_2O 80/20 →

HPLC-FLD

Antioxidant activity → ABTS, FRAP and ORAC →

Counter plate

Determination of phenolic and other polar compounds

PULP

Compounds	Retention time (min)	m/z experimental (M-H)	m/z calculated (M-H)	Fragments	Molecular Formula	Error (ppm)	Score
Perseitol	0.577	211.0829	211.0823		$C_7H_{16}O_7$	2.7	97.5
Quinic acid	0.743	191.0571	191.0561		$C_7H_{12}O_6$	5.7	93.18
Citric acid	1.164	191.02	191.0197	111	$C_6H_8O_7$	1.4	99.04
Succinic acid	1.585	117.0194	118.0194	100	$C_4H_6O_4$	0.93	86.3
Uridine	1.592	243.0625	243.0631	227	$C_9H_{12}N_2O_6$	3.48	96.54
Protocatechuic acid-4-glucoside	3.168	315.0722	315.0722	108, 153	$C_{13}H_{16}O_9$	0.08	99.63
Penstemide	3.81	443.1934	443.1923	101	$C_{21}H_{32}O_{10}$	1.72	98.11
Sinapic acid-hexoside	4.502	385.1143	385.114	223	$C_{17}H_{22}O_{10}$	0.64	99.37
Caffeoylglucose	4.713	341.0886	341.0878	161, 133, 179	$C_{15}H_{18}O_9$	2.36	96.88
Penstemide	5.122	443.1929	443.1923	101, 113, 161	$C_{21}H_{32}O_{10}$	1.13	98.82
Tyrosol-hexoside-pentoside	5.187	431.1562	431.1559	299,119, 113, 137, 131, 149,179, 161	$C_{19}H_{28}O_{11}$	0.85	98.51
p-Coumaric acid glucoside	5.682	325.0935	325.0929	163,145	$C_{15}H_{18}O_8$	1.88	97.47
p-Coumaric acid hexoside pentoside	5.964	457.136	457.1351	145,163	$C_{20}H_{26}O_{12}$	1.75	98.27
p-Coumaric acid glucoside isomer 1	6.053	325.0934	325.0929	145, 117, 119	$C_{15}H_{18}O_8$	1.52	98.59
Feruloylglucoside	6.219	355.1039	355.1035	193,175	$C_{16}H_{20}O_9$	1.3	97.83
p-Coumaric acid rutinoside	6.302	471.1516	471.1508	163, 145	$C_{21}H_{28}O_{12}$	1.72	97.9
Feruloylglucoside isomer 1	6.545	355.1039	355.1035	193,175	$C_{16}H_{20}O_9$	1.17	99.25
Octyl gallate	6.55	281.1393	281.1394		$C_{15}H_{22}O_5$	0.57	98.83
Feruloylquinic acid	7.176	367.1035	367.1035	193	$C_{17}H_{20}O_9$	0.01	98.97
Coumaric acid	7.492	163.0396	163.0401	119	$C_9H_8O_3$	2.26	98.64
Feruloylquinic acid isomer 1	7.580	367.1039	367.1035	193	$C_{17}H_{20}O_9$	0.97	97.56
Feruloylquinic acid isomer 2	7.946	367.1039	367.1035	193	$C_{17}H_{20}O_9$	1.06	99.42
Conduritol F-1-O-(6 -O-E-p-caffeoyl)-d-glucopyranoside	8.195	469.1354	469.1351	179	$C_{21}H_{26}O_{12}$	0.52	98.26

Determination of phenolic and other polar compounds

PULP

Compounds	(mg/100g sample)
Quinic acid	0,16 ± 0,01
Citric acid	3,61 ± 0,19
Succinic acid	1,10 ± 0,09
Protocatechuic acid-4-glucoside	0,24 ± 0,01
Sinapic acid-hexoside	0,74 ± 0,002
Caffeoylglucose	0,98 ± 0,03
Tyrosol-hexoside-pentoside	2,28 ± 0,03
p-Coumaric acid glucoside	8,44 ± 0,15
p-Coumaric acid hexoside pentoside	1,06 ± 0,02
p-Coumaric acid glucoside isomer 1	1,03 ± 0,02
Feruloylglucoside	1,95 ± 0,07
p-Coumaric acid rutinoside	1,64 ± 0,02
Feruloylglucoside isomer1	0,74 ± 0,02
Octyl gallate	0,95 ± 0,02
Feruloylquinic acid	0,77 ± 0,03
Coumaric acid	4,69 ± 0,05
Feruloylquinic acid isomer 1	7,62 ± 0,09
Feruloylquinic acid isomer 2	0,81 ± 0,03
Total	**38,79 ± 0,23**

Determination of phenolic and other polar compounds

PEEL

Compounds	Retention time (min)	m/z experimental (M-H)	m/z calculated (M-H)	Fragments	Molecular Formula	Error (ppm)	Score
Perseitol	0.641	211.0825	211.0823		$C_7H_{16}O_7$	1.0	99.7
Quinic acid	0.791	191.0566	191.0561		$C_7H_{12}O_6$	2.38	98.29
Penstemide	3.914	443.1934	443.1923	101, 113	$C_{21}H_{32}O_{10}$	2.4	97.01
Caffeoylquinic acid	4.296	353.0889	353.0878	191	$C_{16}H_{18}O_9$	2.92	96.31
Procyanidin trimer	4.96	865.2001	865.1985	577, 289	$C_{45}H_{38}O_{18}$	2.28	95.27
Procyanidin dimer I type B	5.049	577.1368	577.1351	289	$C_{30}H_{26}O_{12}$	2.76	94.41
Catechin/Epicatechin	5.597	289.0727	289.0718	123	$C_{15}H_{14}O_6$	2.96	96.98
Procyanidin trimer II	6.001	865.2008	865.1985	577, 289	$C_{45}H_{38}O_{18}$	2.53	94.83
Quercetin-3,4'-diglucoside	6.256	625.1418	625.141	301	$C_{27}H_{30}O_{17}$	1.02	98.86
Procyanidin tetramer type B	6.366	1153.2641	1153.2619	289, 577,865	$C_{60}H_{50}O_{24}$	1.59	96.86
Procyanidin tetramer II type B	6.51	1153.2628	1153.2619	289, 577,865	$C_{60}H_{50}O_{24}$	0.86	99.06
Procyanidin tetramer III type B	6.699	1153.2649	1153.2619	289, 577,865	$C_{60}H_{50}O_{24}$	2.59	89.83
Quercetin-3-O-arabinosyl-glucoside	6.959	595.1308	595.1305	301	$C_{26}H_{28}O_{16}$	0.36	99.42
Procyanidin dimer II type B	8.426	577.1366	577.1351	289	$C_{30}H_{26}O_{12}$	1.74	92.13
Quercetin 3-O-rutinoside/Rutin	9.655	609.1474	609.1461	301	$C_{27}H_{30}O_{16}$	1.98	97.34

Determination of phenolic and other polar compounds

PEEL

Compounds	(mg/100g sample)
Quinic acid	0,50 ± 0,01
Caffeoylquinic acid	189,89 ± 1,07
Procyanidin trimer	93,90 ± 3,01
Procyanidin dimer I type B	612,71 ± 3,62
Catechin/epicatechin	516,81 ± 3,04
Procyanidin trimer II	467,51 ± 2,73
Quercetin-3,4'-diglucoside	270,05 ± 2,19
Procyanidin tetramer type B	166,23 ± 2,43
Procyanidin tetramer II type B	321,60 ± 3,27
Procyanidin tetramer III type B	204,86 ± 1,45
Quercetin-3-O-arabinosyl-glucoside	19,76 ± 0,22
Procyanidin dimer II type B	51,44 ± 0,36
Quercetin 3-O-rutinoside/Rutin	35,31 ± 0,61
Total	**2950,59 ± 2,13**

Determination of phenolic and other polar compounds

SEED

Compounds	Retention time (min)	m/z experimental (M-H)	m/z calculated (M-H)	Fragments	Molecular Formula	Error (ppm)	Score
Perseitol	0.872	211.0827	211.0823	101	$C_7H_{16}O_7$	1.88	99.01
Quinic acid	0.917	191.0567	191.0561	108	$C_7H_{12}O_6$	4.05	93.95
Citric acid	1.183	191.0197	191.0197	111, 101, 113	$C_6H_8O_7$	0.04	99.88
Hydroxytyrosol 1-glucoside	3.126	315.1094	315.1085	135, 153	$C_{14}H_{20}O_8$	2.48	96.57
Caffeoylquinic acid	3.585	353.088	353.0878	191, 135	$C_{16}H_{18}O_9$	0.55	99
Salidroside or Tyrosol α-(β-D-glucopyranoside)	4.012	299.1138	299.1136	119	$C_{14}H_{20}O_7$	0.43	99.42
Procyanidin dimer	4.2	577.1358	577.1351	289	$C_{30}H_{26}O_{12}$	1.03	97.94
Penstemide	4.344	443.1924	443.1923	101	$C_{21}H_{32}O_{10}$	0.18	99.74
3-O-p-Coumaroylquinic acid	4.538	337.093	337.0929	163	$C_{16}H_{18}O_8$	0.27	99.8
Catechin/Epicatechin	4.931	289.0726	289.0718	123	$C_{15}H_{14}O_6$	2.64	97.27
Caffeoylquinic acid	5.075	353.0887	353.0878	135, 191	$C_{16}H_{18}O_9$	2.54	97.08
Procyanidin tetramer type A	5.357	1151.2477	1151.2463	577,289	$C_{60}H_{48}O_{24}$	1.16	98.17
Procyanidin dimer type B	5.512	577.1355	577.1351	289	$C_{30}H_{26}O_{12}$	0.47	99.09
Procyanidin trimer type A I	5.922	863.1852	863.1829	289	$C_{45}H_{36}O_{18}$	2.69	94.25
Catechin/Epicatechin	6.093	289.0726	289.0718	123	$C_{15}H_{14}O_6$	2.77	95.5
Procyanidin trimer type A II	6.315	863.1843	863.1829	289	$C_{45}H_{36}O_{18}$	2.08	95.34
Vanillic acid glucoside	6.366	329.0878	330.0951	123, 167	$C_{14}H_{18}O_9$	0.14	99.06
(1'S, 6'R)-8'-Hydroxyabscisic acid beta-D-glucoside	8.546	441.177	441.1766	330,139	$C_{21}H_{30}O_{10}$	0.67	99.24

Determination of phenolic and other polar compounds

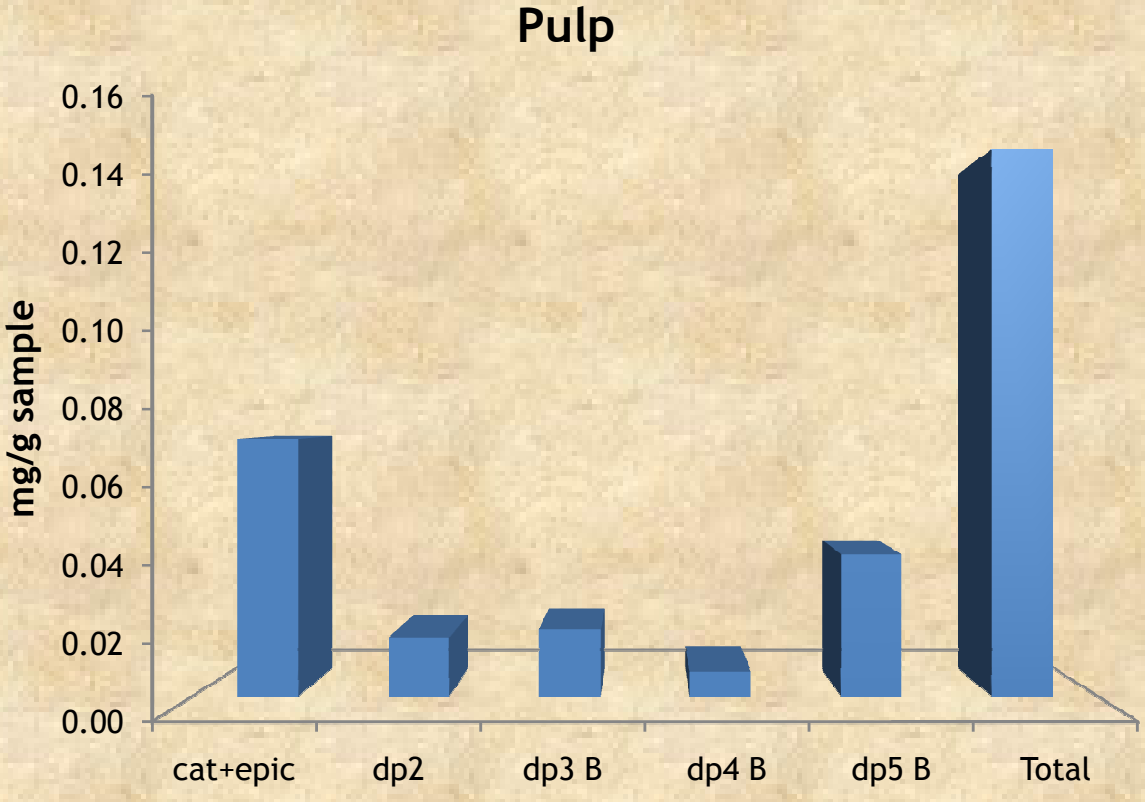
SEED

Compounds	(mg/100g sample)
Quinic acid	0,10 ± 0,001
Citric acid	4,63 ± 0,14
Hydroxytyrosol 1-glucoside	38,95 ± 0,61
Caffeoylquinic acid	112,29 ± 0,41
Salidroside or Tyrosol α-(ß-D-glucopyranoside)	223,66 ± 1,33
Procyanidin dimer	28,28 ± 0,15
3-O-p-coumaroylquinic acid	7,01 ± 0,05
Catechin/Epicatechin	84,24 ± 2,10
Caffeoylquinic acid	6,69 ± 0,05
procyanidin tetramer type A	97,60 ± 1,10
procyanidin dimer type B	51,58 ± 0,98
procyanidin trimer type A I	150,64 ± 1,31
Catechin/Epicatechin	130,31 ± 1,25
Procyanidin trimer type A II	170,67 ± 1,90
Vanillic acid glucoside	6,74 ± 0,27
Total	**1113,39 ± 0,76**

Antioxidant Activity

	ABTS (mmol eq trolox/100g sample)	FRAP (mmol eq $FeSO_4$/100g sample)	ORAC (µmol eq trolox/100g sample)
Seed	46.97 ± 0.18	2886.13 ± 26.59	44.19 ± 0.64
Peel	168.32 ± 14.74	3800.87 ± 37.38	100.92 ± 4.12
Pulp	1.87 ± 0.02	50.53 ± 0.51	6.36 ± 0.12

Determination of Proanthocyanidins



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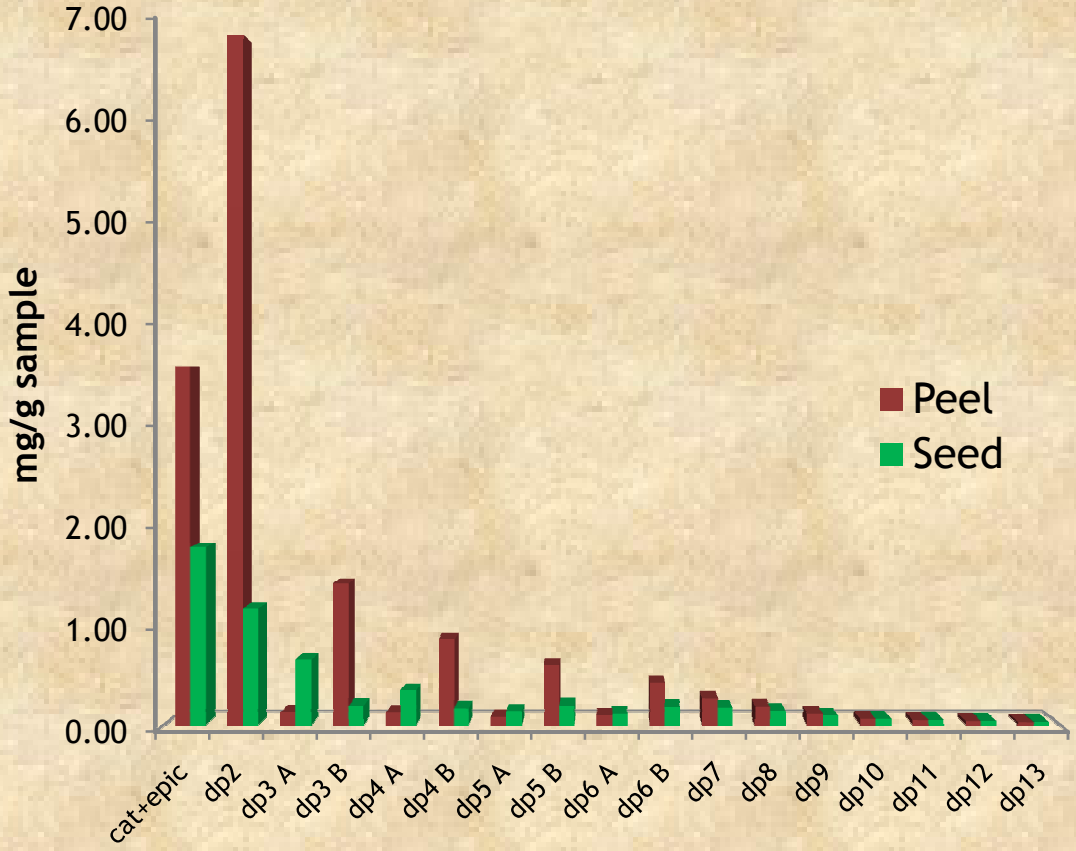

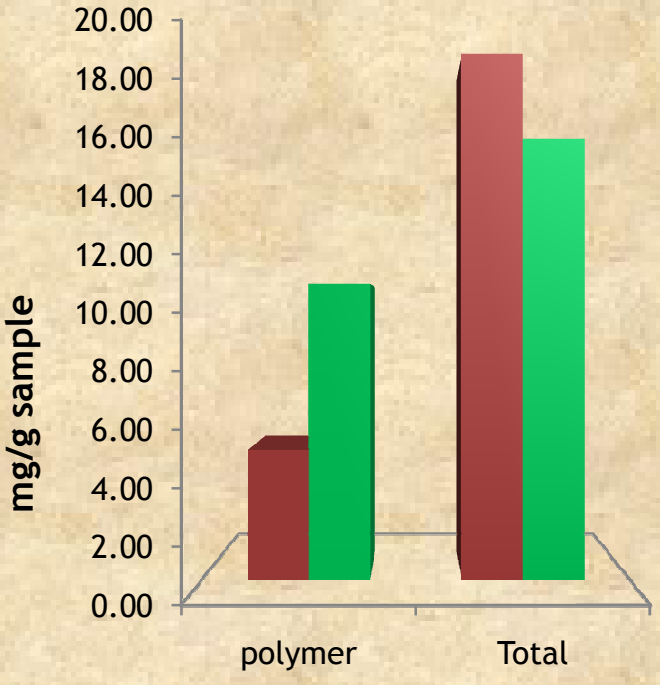

Conclusions

1. Avocado pulp is a good source of phenolic acids
2. Avocado fruit by-products, as peel and seed, contain high amounts of flavan-3-ols
3. Procyanidins type A are predominant in avocado seed, whereas procyanidins type B are prevalent in avocado peel
4. Avocado peel and seed could be used as raw material for the production of nutraceuticals and/or food ingredients

Thank you for your attention!

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