



Bioactive phytochemicals in apple cultivars from the Portuguese protected geographical indication “Maçã de Alcobaça:” Basis for market segmentation

Domingos P. F. Almeida, Maria S. Gião, Manuela Pintado & Maria Helena Gomes

To cite this article: Domingos P. F. Almeida, Maria S. Gião, Manuela Pintado & Maria Helena Gomes (2017) Bioactive phytochemicals in apple cultivars from the Portuguese protected geographical indication “Maçã de Alcobaça:” Basis for market segmentation, *International Journal of Food Properties*, 20:10, 2206-2214, DOI: [10.1080/10942912.2016.1233431](https://doi.org/10.1080/10942912.2016.1233431)

To link to this article: <https://doi.org/10.1080/10942912.2016.1233431>



© 2017 Taylor & Francis Group, LLC



Accepted author version posted online: 19 Oct 2016.
Published online: 19 Jan 2017.



Submit your article to this journal [↗](#)



Article views: 1012



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 1 View citing articles [↗](#)



Bioactive phytochemicals in apple cultivars from the Portuguese protected geographical indication “Maçã de Alcobaça:” Basis for market segmentation

Domingos P. F. Almeida ^a, Maria S. Gião^b, Manuela Pintado ^b, and Maria Helena Gomes^b

^aInstituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal; ^bCBQF - Centro de Biotecnologia e Química Fina, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino Almeida, 4200-072 Porto, Portugal

ABSTRACT

Phytochemical composition and antioxidant activity of eight apple (*Malus domestica*) cultivars (“Casa Nova,” “Fuji,” “Golden Delicious,” “Granny Smith,” “Jonagored,” “Reinette Grise,” “Royal Gala,” and “Starking”) from the “Maçã de Alcobaça” protected geographic indication, Portugal, were characterized to support market segmentation based on phytochemical content. Total phenolics, flavonoids, and anthocyanin contents, individual polyphenols and antioxidant activity were assessed in the flesh and skin of fruit at the ripe stage. A market segmentation of apple cultivars based on these bioactive phytochemicals is proposed. Fruit eaten with the peel can be segmented in three classes: (1) the flavonoid-rich “Starking,” “Reineta,” “Galaxy,” “Casa Nova,” “Jonagored;” (2) the quercetin-rich “Fuji,” “Galaxy,” “Casa Nova;” and (3) flavonol and procyanidin-rich “Starking,” “Reinette,” “Jonagored,” “Casa Nova.” When apples were peeled before consumption, two phytochemical classes were proposed: (1) the flavonoid-rich “Reinette” and “Casa Nova,” and the (2) chlorogenic acid-rich “Reineta,” “Casa Nova,” and “Starking.”

ARTICLE HISTORY

Received 26 April 2016
Accepted 3 September 2016

KEYWORDS

Ascorbic acid; Antioxidants;
Flavonoid; *Malus domestica*;
Polyphenols; Phytochemicals

Introduction

European consumers expect stores to display several apple cultivars year-round. Easy to prepare, convenient to consume, and inexpensive, apple is a fruit compatible with modern lifestyles. Not surprisingly, apple account for a significant proportion of total fruit in the European diet, with estimated per capita availability of about 19 kg.^[1] About 25 apple cultivars are produced in the European Union in volumes exceeding 10,000 tones. Among these, “Golden Delicious,” “Gala,” and “Idared” are the prevailing types.^[2]

Despite the differences in apple consumption habits among European countries,^[2,3] the overall intake of apple in the European diet is aligned with recommendations. Due to the combination of composition, intake amounts, and consumption frequency, apple is a major source of bioactive phenolics in the European diet.^[1] The frequent consumption of apple has been associated to the reduction of certain types of cancer, cardiovascular diseases, and diabetes.^[4–7] More specifically, the consumption of one or more apples a day has been suggested to reduce the risk of malignant neoplasms of the oral cavity and pharynx, esophagus, colorectum, breast, ovary, and prostate,^[8] and the intake of five apples per week can improve lung function.^[9]

The health benefits of apple have been attributed to phenolic phytochemicals and also to ascorbic acid.^[10] Compounds in each one of these chemical classes contribute to the strong antioxidant activity of apple.^[11] Phenolic compounds comprise several subclasses synthesized by different branches of the phenylpropanoid pathway. Three subclasses of bioactive phenolic compounds are relevant in apple: hydroxycinnamic acids,

flavonoids, and dihydrochalcones.^[12,13] Flavan-3-ols, anthocyanidins, and flavonols are important groups among the apple flavonoids. Apple flavonols include several quercetin glycosides and derivatives, which are dietary components with high anti-inflammatory and antioxidant properties.^[14] Phenolic acids and flavonoids are also relevant as they determine fruit quality characteristics such as color, flavor, bitterness, and astringency.^[15] Concentration of these bioactive phytochemicals in apples varied considerably among cultivar and tissue type.^[10,16–18] Although apple peel accounts for a small percentage of whole fruit weight, its richness in phytochemicals can contribute to additional health benefits. The concentration of phenolic compounds, and, therefore, antioxidant activity and bioactivity, are higher in the peel than in the flesh.^[11,19]

The European Union recognizes 1214 food products with protected designations of origin (PDO) or protected geographical indications (PGI), whose attributes are related to the territory. Of these, 343 or 28.3% are fruits, vegetables, and cereals, including more than 20 apples.^[20] “Maçã de Alcobaça” is a Portuguese PGI with a pomological tradition going back to the Middle Ages and remains one of the main apple growing regions in the country. However, the phytochemical content of apples from the “Maçã de Alcobaça” PGI remained uncharacterized.

This study reports the phytochemical content and antioxidant capacity of the peel and flesh of eight apple cultivars from the PGI of “Maçã de Alcobaça,” Portugal. These results improve our knowledge of *terroir*-specific nutritional composition and support a proposal of market segmentation of apple cultivars based on differences in their phytochemical composition.

Materials and methods

Fruit material and sample preparation

Eight apple (*Malus × domestica*) cultivars—“Casa Nova,” “Fuji,” “Golden Delicious,” “Granny Smith,” “Jonagored,” “Reinette Grise,” “Royal Gala,” and “Starking,”—grown in the “Maçã de Alcobaça” PGI region, were analyzed. Cultivar classification based on peel color and taste is presented in Table 1. Fruits harvested at commercial maturity were stored at 0°C, 90–95% relative humidity (RH) for 3.0 to 3.5 months before sampling. Fruits with uniform shape and size, with the average mass indicated in Table 1, and no surface damage were selected for analyses. A sample of 25 fruit were cored and peeled to obtain the edible flesh, peel, and core (Table 1).

Tissue cylinders with 14 mm diameter were excised from the equatorial region of the fruit. The peel layer (1 mm thick) was removed with a scalpel and the tissue located beneath the peel was used for flesh analysis. The tissue was immediately frozen in liquid nitrogen and subsequently ground in a mortar with silica sand (1 g). This preparation procedure lasted less than 60 s.

Chemicals

Chemicals and solvents were of analytical grade or higher. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; ABTS) diammonium salt, methanol, acetone, and formic acid were purchased from

Table 1. Classification average fruit size and proportion of fruit parts of the apple cultivars included in the study.

Cultivar	Color class	Taste class	Mass (g)	Pulp (%)	Peel (%)	Core (%)
“Casa Nova”	Bicolor	Acid	98	76	11	13
“Fuji”	Bicolor	Sweet	140	78	12	10
“Galaxy”	Bicolor	Sweet	135	81	9	10
“Golden Delicious”	Yellow	Sweet	150	80	10	10
“Granny Smith”	Green	Acid	149	80	10	10
“Jonagored”	Bicolor	Sweet	156	81	9	10
“Reinette”	Russet	Acid	157	82	10	8
“Starking”	Red	Sweet	129	79	10	11
Average	—	—	139	80	10	10

Note: Values are means of 25 fruits.

Sigma–Aldrich (St. Louis, MO, USA). The standards of ascorbic acid, gallic acid, chlorogenic acid, quercetin, quercitrin, rutin, hyperin, isoquercitin, (+)-catechin, (–)-epicatechin, procyanidin B₁, procyanidin B₂, and phloridzin were also provided by Sigma–Aldrich (St. Louis, MO, USA). Folin–Ciocalteu's reagent and hydrochloric acid were obtained from Merck (Damstadt, Germany).

Antioxidant activity

Samples were sequentially extracted with acidified methanol (1% HCl) and acetone. Total antioxidant activity was measured by the oxidation of the radical cation ABTS diammonium salt^[21] and expressed as ascorbic acid equivalents on a fresh weight basis. All tested samples were replicated six times.

Ascorbic acid content

Total ascorbic acid was determined as described by the EN 14130:2003. Ascorbic acid was extracted from a test sample with a solution of metaphosphoric acid. L-cysteine was used to reduce dehydroascorbic acid to ascorbic acid and total ascorbic acid was quantified by high-performance liquid chromatography (HPLC; Waters Series 600, Massachusetts, USA) with detection at 265 nm.

Total phenolic compounds

Total phenolics were extracted in methanol acidified with 1% HCl. The total concentration of phenolic compounds was determined by the Folin–Ciocalteu method^[22] with gallic acid as a standard.

Total flavonoids and anthocyanins

Total flavonoids in the peel and flesh were determined in the acidified methanolic extract. The reaction was carried out as described^[23] and quantification of total flavonoids performed by spectrophotometry at 510 nm using rutin as a standard. The content on anthocyanins was measured in methanolic extract of peel and flesh. The concentration of total anthocyanins was determined spectrophotometrically at 530 nm (Shimadzu 1240 ultraviolet [UV]–visible, Tokyo, Japan) by pH-differential method.^[24] The anthocyanin index (A530 nm) is expressed per gram of fresh mass.

Identification and quantification of individual phenolics

Individual phenolic compounds were separated and quantified by HPLC–diode array detector (DAD; Waters Series 600, Massachusetts, USA) as described.^[25] Compounds were separated in a 5 µm C18 column (4.6 mm × 250 mm) eluted at a flow rate of 0.75 mL min⁻¹. Water, methanol, and formic acid in the proportions 92.5:5:2.5 and 5:92.5:2.5 were used as eluent A and B, respectively. Solvent B increased from 0 to 20% in 10 min, 20 to 50% in the subsequent 50 min, and reduction from 50 to 0% in 10 min. Detection was performed at 280 and 320 nm. All samples were run in duplicate. Phenolic substances were identified based on retention time of the pure substance and calibration curves were established for quantification.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) with cultivar as a fixed factor. When the *F*-test revealed significant differences among cultivars, means were separated by the Duncan multiple range test at $\alpha = 0.05$. Pearson correlation coefficients between the variables were calculated. All statistical analyses were performed with SPSS software (v. 20, Chicago IL, USA).

Results and discussion

Characteristics of apple cultivars

Graded apples of average market size were used for phytochemical characterization. With the exception of the smaller fruit of the landrace cultivar “Casa Nova,” which weighed less than 100 g each, the average apple weight ranged from 129 g in “Starking” to 156 or 157 g in “Jonagored” and “Reinette,” respectively (Table 1). The color class is a readily observed genotypic characteristic (Table 1). Cultivars were also classified into two taste groups based on ratio between their soluble solids content (SSC) and titratable acidity (values not shown). The average ratio between SSC and titratable acidity for the eight cultivars was 39. Cultivars were considered “sweet” or “sour” if their sugar to acid ratio was above or below the average, respectively.

Apples are eaten with or without peel. This consumer preference has significant consequences upon phytochemical intake. The inedible core and stem accounted for 10% of the fruit mass (Table 1), the same proportion considered in the USDA nutrient database^[26] for unpeeled apple. The peel accounted for *ca.* 10% of the fruit mass (Table 1), a value slightly lower than the 13% reported elsewhere.^[26] If the core is neglected, the peel accounts for 11% of the edible fruit mass and the flesh for 89%.

Antioxidant activity of methanol and acetone extracts

The antioxidant activity was evaluated separately in apple peel and flesh and in extracts obtained with two solvents with different polarities. Given the existence of water-soluble (e.g., phenolic compounds and ascorbic acid) and lipid-soluble antioxidant compounds (e.g., carotenoids and tocopherols), antioxidant activity was measured in a more polar methanolic and in a nonpolar acetone extracts. The average (eight cultivars) antioxidant activity was higher in the methanolic than in the acetone extract, 10- and 5-fold higher in the flesh and peel, respectively (Table 2).

The ratio between the antioxidant activity of peel and flesh in the methanolic extract was cultivar-dependent, ranging 3.3 to 3.8 in “Reinette,” “Granny Smith,” and “Golden Delicious,” up to 9 in “Starking.” The higher antioxidant activity of the apple peel in relation to the flesh is consistent with results reported elsewhere.^[27] Antioxidant activity in the flesh and peel varied 3-fold among cultivars. Flesh antioxidant activity ranged from the 0.34 mg g⁻¹ in “Fuji” to 1.06 mg g⁻¹ in “Reinette,” whereas in the peel it ranged from 1.78 mg g⁻¹ in “Granny Smith” to 5.49 mg g⁻¹ in “Starking” (Table 2). No direct correlation was observed between the cultivar color class (Table 1) and antioxidant activity of flesh and peel. For instance, the peel of red “Fuji” and the bicolored “Galaxy” had similar levels of antioxidant activity to the yellow “Golden Delicious” and the green “Granny Smith.”

Table 2. Antioxidant activity of extracts of the peel and the flesh of eight apple cultivars obtained by two solvents with different polarities.

Cultivar	Antioxidant activity (mg g ⁻¹)			
	Methanolic extract		Acetone extract	
	Flesh	Peel	Flesh	Peel
“Casa Nova”	0.77 ± 0.08 ^b	3.27 ± 0.18 ^b	0.14 ± 0.03 ^a	0.59 ± 0.06 ^{ab}
“Fuji”	0.34 ± 0.02 ^e	1.98 ± 0.06 ^{cd}	0.05 ± 0.00 ^b	0.65 ± 0.03 ^{ab}
“Galaxy”	0.54 ± 0.03 ^{cd}	2.93 ± 0.32 ^{bcd}	0.03 ± 0.01 ^b	0.42 ± 0.05 ^{bc}
“Golden Delicious”	0.48 ± 0.00 ^{de}	1.82 ± 0.47 ^{cd}	0.03 ± 0.00 ^b	0.21 ± 0.03 ^c
“Granny Smith”	0.54 ± 0.04 ^{cd}	1.78 ± 0.13 ^d	0.05 ± 0.01 ^b	0.57 ± 0.06 ^{ab}
“Jonagored”	0.65 ± 0.02 ^{bc}	3.00 ± 0.52 ^{bc}	0.09 ± 0.04 ^{ab}	0.58 ± 0.06 ^{ab}
“Reinette”	1.06 ± 0.05 ^a	3.51 ± 0.30 ^b	0.07 ± 0.04 ^{ab}	0.83 ± 0.12 ^a
“Starking”	0.61 ± 0.07 ^{cd}	5.49 ± 0.59 ^a	0.02 ± 0.01 ^b	0.79 ± 0.23 ^a
Average	0.62 ± 0.08	2.97 ± 0.43	0.06 ± 0.01	0.58 ± 0.07

Note: Values are mean ± SE (*n* = 3).

Means followed by the same letter within columns are not significantly different at $\alpha \leq 0.05$.

Ascorbic acid concentration

The average concentration of total ascorbic acid in the eight apple cultivars was 31 mg kg⁻¹ (Table 3), ranging from the lowest value of 23 mg kg⁻¹ in “Galaxy” to the highest level in “Reinette” (133 mg kg⁻¹). These values are, on average, slightly lower than the 40 and 46 mg kg⁻¹ reported for peeled and unpeeled apples of unspecified cultivars, respectively.^[26]

Total phenolic content

Total phenolic concentration in the peel was, on average, 5-fold higher than that in the flesh. “Reinette,” “Jonagored,” and “Casa Nova” were the cultivars with higher phenolic concentrations in the flesh, whereas, “Starking,” “Reinette,” and “Casa Nova” had the higher concentrations in the peel (Table 3). Higher phenolic concentrations in the peel than in the flesh has been documented for several apple cultivars and other fruit types.^[26–29]

Total phenolic content in the flesh ranged 3.8-fold from 5.2 mg g⁻¹ in “Granny Smith” to 14.4 mg g⁻¹ in “Reinette.” Peel phenolic content ranged 4.4-fold, from 14.7 mg g⁻¹ in “Fuji” to 65.1 mg g⁻¹ in “Starking” (Table 3). Total phenolic content in the peel was positively correlated with antioxidant activity but not related with the color class. These results highlight the importance of apple peel as a dietary source of phenolics.

Flavonoid compounds

Flavonoids account for about 60% of the total dietary polyphenols.^[30] Total flavonoids were more abundant in the peel than in the pulp in every apple cultivar, a difference that ranged from 4- to 10-fold (Table 3). The highest concentration of flavonoids was measured in the peel of “Starking” (14.5 mg g⁻¹) and the lowest in “Granny Smith” (5.7 mg g⁻¹). However, no direct relationship was observed between peel flavonoid concentration and the cultivar color class (Table 1); for instance, similar flavonoid levels were measured in the bicolor “Fuji,” the yellow “Golden Delicious,” and the green “Granny Smith.” Flesh flavonoid levels were highest in “Reinette” (3.3 mg g⁻¹) and lowest in “Golden Delicious” (1.2 mg g⁻¹).

Flavonoids can be divided into subclasses based on their molecular structure. Compounds from three of flavonoid subclasses were identified in the apple cultivars analyzed: flavonols, anthocyanins, and flavanols. The flavonol quercetin and four quercetin glycosides were identified in the peel of apple fruit from all cultivars except “Reinette” (Table 5). Quercetin was also identified in the flesh of apple cultivars, with an average concentration of 0.2 mg kg⁻¹ (not shown), but was much more abundant in the peel. Quercetin glycosides were not detected in the flesh of the any of the cultivars analyzed. Hyperin, a quercetin-3-galactoside, was the most abundant quercetin derivative in apple skin, followed by quercitrin

Table 3. Total ascorbic acid, phenolics, flavonoid, and anthocyanins concentration in eight apple cultivars.

Cultivar	Ascorbic acid (mg kg ⁻¹)	Total phenolics (mg g ⁻¹)		Total flavonoids (mg g ⁻¹)		Peel anthocyanins (A ₅₃₀)
		Flesh	Peel	Flesh	Peel	
“Casa Nova”	37 ± 9 ^b	10.8 ± 0.9 ^{ab}	52.9 ± 7.4 ^{abc}	2.2 ± 0.2 ^b	9.3 ± 0.5 ^{bcd}	0.197 ± 0.035 ^{de}
“Fuji”	30 ± 0 ^b	9.1 ± 0.6 ^{bc}	14.7 ± 0.4 ^f	0.6 ± 0.1 ^d	6.4 ± 0.5 ^{de}	0.654 ± 0.037 ^c
“Galaxy”	23 ± 3 ^b	7.1 ± 0.9 ^{bc}	49.2 ± 1.4 ^{bcd}	3.3 ± 0.1 ^a	11.8 ± 0.9 ^{ab}	1.151 ± 0.147 ^b
“Golden Delicious”	30 ± 0 ^b	5.6 ± 0.2 ^c	36.2 ± 4.2 ^{de}	1.3 ± 0.1 ^c	9.7 ± 0.7 ^{bc}	0.033 ± 0.007 ^e
“Granny Smith”	27 ± 7 ^b	5.2 ± 0.3 ^c	25.3 ± 2.6 ^{ef}	1.2 ± 0.0 ^c	7.9 ± 0.7 ^{cde}	0.037 ± 0.007 ^e
“Jonagored”	33 ± 3 ^b	11.2 ± 0.3 ^{ab}	42.3 ± 4.8 ^{cd}	1.3 ± 0.1 ^c	5.7 ± 0.5 ^e	0.315 ± 0.077 ^d
“Reinette”	133 ± 29 ^a	14.4 ± 0.8 ^a	61.3 ± 5.1 ^{ab}	1.5 ± 0.2 ^c	9.1 ± 1.3 ^{bcd}	0.054 ± 0.005 ^{de}
“Starking”	37 ± 3 ^b	7.2 ± 0.8 ^{bc}	65.1 ± 3.6 ^a	1.6 ± 0.2 ^c	14.5 ± 1.9 ^a	1.773 ± 0.167 ^a
Average	46 ± 15	8.8 ± 0.1	43.4 ± 6.2	1.6 ± 0.3	9.3 ± 1.0	0.527 ± 0.224

Values are mean ± SE (n = 3).

Means followed by the same letter within columns are not significantly different at α ≤ 0.05.

(quercetin 3-rhamnoside) and isoquercetin (quercetin-3-glucoside), present in similar concentrations. Rutin, a quercetin-rutinoside, was present in the peel in concentrations of the same order of magnitude of those of quercetin. When the sum of quercetin and quercetin glycosides is considered, “Casa Nova,” “Fuji,” and “Galaxy” were quercetin-rich cultivars, whereas “Golden Delicious,” “Granny Smith,” and “Starking” had lower levels of quercetin glycosides (Table 4). “Reinette” had the highest flavonoid concentration in the peel (Table 3) but low levels of quercetin glycosides (Table 4) and anthocyanins (Table 3), suggesting that other flavonoids are abundant in this cultivar.

The flavanols catechin and epicatechin were concentrated in the peel. “Starking,” “Reinette,” and “Casa Nova” were rich in these flavanols, which were less concentrated in “Golden Delicious” and “Granny Smith” (Table 4). Procyanidins were also found on the peel of all apple cultivars. Procyanidin B2, a dimer of epicatechin, was more abundant than procyanidin B1, a dimer formed from epicatechin and catechin. A significant correlation was observed between the flavonol (catechin and epicatechin) and procyanidin concentrations in the apple cultivars characterized in this study (Table 4). Phloridzin is a glycosylated dihydrochalcone abundant in apple peel. Phloridzin was more abundant in “Reinette” than in “Galaxy,” “Golden Delicious,” and “Fuji” (Table 4).

Anthocyanins are a class of flavonoids responsible for the reddish color of some apple cultivars. Therefore, in contrast with total phenolics or flavonoids, differences in peel anthocyanin content were related to color. Anthocyanins were absent or detected at very low levels in the flesh of any of the eight apple cultivars analyzed. However, a 54-fold difference was measured in the peel of different cultivars, ranging from the lowest index of 0.033 to 0.037 in “Golden Delicious” and “Granny Smith” to the highest indices of 1.773 in “Starking” (Table 3). Idaein or cyanidin-3-galactoside is the major anthocyanin in apple skin.^[31]

The contribution of apple to the dietary intake of flavonoids in the European diet is significant. For instance, apple is the top ranking source of flavonoids for the Spanish population, outranking red wine.^[32] In addition, apple is a top ranking food source of flavonoids from each one of these subclasses: flavanols, flavan-3-ols, proanthocyanidin, and anthocyanidins.^[32]

Chlorogenic acid

Phenolic acids are a subclass of phenolic compounds which accounts for *ca.* 30% of the dietary polyphenols.^[30] Chlorogenic acid is the major phenolic acid in apple and the only phenolic compound that is more abundant in the flesh than in the peel (Table 5). “Reinette,” “Casa Nova,” and “Starking” had flesh concentrations of chlorogenic acid higher than 1 g kg⁻¹, whereas “Granny Smith” had only 0.1 g kg⁻¹ (Table 5).

Segmentation of apple cultivars based on phytochemical composition

The results presented herein show that the conventional market segmentation of apple cultivars based on skin color does not coincide with a nutritional and phytochemical segmentation. This lack of coincidence was observed for total phenolics and flavonoids. Anthocyanin presence, although directly related to red hues, is also highly variable within the red and bicolored cultivars.

In addition to the market categories based on color and taste, apple cultivars from the PGI “Maçã de Alcobça” can be segmented based on their phytochemical content (Table 6). Four categories are proposed based on the major apple phytochemicals taking into account the consumer preference for eating the apple peel. This is a relevant consumption preference since most apple phytochemicals were more concentrated in the peel than in the flesh. “Reinette” is a particular case of a cultivar whose peel is rich in several phytochemicals, but due to the intense russetting this apple cultivar is often peeled.

Three classes are proposed for apples eaten with peel: (1) the flavonoid-rich “Starking,” “Reineta,” “Galaxy,” “Casa Nova,” “Jonagored;” (2) the quercetin-rich “Fuji,” “Galaxy,” “Casa Nova;” and (3) flavonol and procyanidin-rich “Starking,” “Reinette,” “Jonagored,” and “Casa Nova.” Peeled apples can be categorized into two phytochemical classes: (1) the flavonoid-rich “Reinette” and “Casa Nova;” and (2) the chlorogenic acid-rich “Reineta,” “Casa Nova,” and “Starking.” Segmentation

Table 4. Individual flavonoids in the peel of the eight apple cultivars.

Cultivar	Quercetin (mg kg ⁻¹)	Quercitrin (mg kg ⁻¹)	Rutin (mg kg ⁻¹)	Hyperin (mg kg ⁻¹)	Isoquercitin (mg kg ⁻¹)	Catechin (mg kg ⁻¹)	Epicatechin (mg kg ⁻¹)	Procyanidin B1 (mg kg ⁻¹)	Procyanidin B2 (mg kg ⁻¹)	Phloridzin (mg kg ⁻¹)
"Casa Nova"	10.3±1.0 ^a	422.9 ± 23.3	7.0 ± 2.4 ^{abc}	707.1 ± 1.3 ^{bc}	381.8 ± 5.3 ^a	280.4 ± 51.7 ^{bcd}	931.5 ± 79.7 ^b	139.1 ± 25.9 ^b	532.8 ± 48.5 ^{cd}	380.7 ± 33.8 ^c
"Fuji"	9.1±2.6 ^a	337.1 ± 3.7	10.6 ± 2.2 ^a	1007.7 ± 0.7 ^a	365.2 ± 6.6 ^a	186.3 ± 1.9 ^{de}	818.1 ± 152.6 ^{bc}	58.8 ± 0.1 ^d	443.5 ± 3.4 ^d	168.1 ± 0.3 ^f
"Galaxy"	10.6±1.1 ^a	259.0 ± 106.7	8.2 ± 2.9 ^{abc}	923.6 ± 118.3 ^{ab}	280.3 ± 113.4 ^{abc}	204.5 ± 27.6 ^d	804.6 ± 133.9 ^{bc}	67.0 ± 11.8 ^{cd}	465.7 ± 28.1 ^d	131.4 ± 52.3 ^g
"Golden Delicious"	6.1±2.6 ^{ab}	182.9 ± 1.5	9.2 ± 1.7 ^{ab}	372.6 ± 1.5 ^f	114.7 ± 0.2 ^c	170.6 ± 3.1 ^f	603.0 ± 2.2 ^c	49.7 ± 1.9 ^e	381.5 ± 1.6 ^e	182.8 ± 5.4 ^e
"Granny Smith"	2.3±0.5 ^b	252.4 ± 3.5	3.3 ± 0.6 ^{bc}	568.3 ± 0.6 ^d	228.5 ± 0.8 ^{ab}	133.9 ± 14.2 ^g	624.5 ± 42.8 ^c	59.4 ± 2.9 ^d	280.7 ± 15.6 ^f	220.3 ± 9.6 ^d
"Jonagored"	7.6±1.6 ^{ab}	286.5 ± 2.7	2.4 ± 0.7 ^c	453.7 ± 5.6 ^e	111.2 ± 1.7 ^c	291.4 ± 2.0 ^c	1001.2 ± 12.4 ^b	92.9 ± 2.1 ^c	588.3 ± 0.7 ^c	342.4 ± 1.2 ^c
"Reinette"	ND	ND	ND	ND	ND	325.2 ± 1.6 ^b	1199.3 ± 185.1 ^b	148.3 ± 0.2 ^b	771.8 ± 5.2 ^b	2054.9 ± 11.8 ^a
"Starking"	12.4±3.1 ^a	123.8 ± 7.4	4.2 ± 1.7 ^{bc}	443.7 ± 4.7 ^e	58.7 ± 26.7 ^{cd}	473.7 ± 0.2 ^a	2126.2 ± 3.1 ^a	179.3 ± 0.0 ^a	1323.7 ± 3.5 ^a	746.0 ± 0.3 ^b
Average	7.3±1.5	266.4 ± 97.9	5.6 ± 1.3	639.5 ± 248.4	220.1 ± 129.0	258.3 ± 109.4	1013.5 ± 490.1	99.3 ± 49.5	598.5 ± 327.4	572.3 ± 686.5

Values are mean ± SE (n = 3).

Means followed by the same letter within columns are not significantly different at α ≤ 0.05.

ND: not detected.

Table 5. Chlorogenic acid concentration in the flesh and peel of the eight apple cultivars.

Cultivar	Chlorogenic acid (mg kg ⁻¹)	
	Flesh	Peel
"Casa Nova"	3284.0 ± 9.7 ^b	276.4 ± 7.3 ^b
"Fuji"	668.4 ± 2.4 ^e	253.6 ± 0.3 ^b
"Galaxy"	580.8 ± 1.3 ^f	191.7 ± 10.9 ^c
"Golden Delicious"	736.8 ± 5.4 ^d	68.6 ± 1.8 ^d
"Granny Smith"	134.0 ± 1.0 ^g	0.0 ± 0.0 ^g
"Jonagored"	572.9 ± 2.2 ^f	49.7 ± 1.2 ^e
"Reinette"	4161.5 ± 44.5 ^a	516.7 ± 3.5 ^a
"Starking"	1790.2 ± 37.7 ^c	19.1 ± 0.8 ^f
Average	1491.1 ± 1388.1	172.0 ± 175.4

Values are mean ± S.E. ($n = 3$).

Means followed by the same letter within columns are not significantly different at $\alpha \leq 0.05$.

Table 6. Proposed market segmentation of apple cultivars based on their phytochemical content.

Phytochemical class	Consumption	
	With peel	Without peel
Flavonoid-rich	"Starking," "Reinette," "Galaxy," "Casa Nova," Jonagored"	"Reinette," "Casa Nova"
Quercetin-rich	"Fuji," "Galaxy," "Casa Nova"	—
Chlorogenic acid	—	"Reinette," "Casa Nova," "Starking"
Flavonol and procyanidin-rich	"Starking," "Reinette," "Jonagored," "Casa Nova"	—

based on anthocyanin content, an important subclass of flavonoids, can be easily evaluated by the consumers based on the intensity of the red skin color.

Conclusions

The highest concentration of phytochemicals is located in the apple skin instead of the flesh. The market segmentation of apple cultivars based on skin color is not related with their overall phytochemical content. The present study documents the compositional differences in apple cultivars and proposes a phytochemical classification of apple cultivars from the PGI "Maçã de Alcobaça."

ORCID

Domingos P. F. Almeida  <http://orcid.org/0000-0001-8429-8585>

Manuela Pintado  <http://orcid.org/0000-0002-0760-3184>

References

- [1] Martinez-Palou, A.; Rohner-Tielen, E. Fruit and Vegetables. Fresh and Healthy on European Tables. Eurostat, Statistics in Focus **2008**, 60, 8.
- [2] USDA. EU-28 Fresh Deciduous Fruit Annual Production in 2013 to Rebound . GAIN Report Number AU1309, USDA Foreign Agricultural Service, Washington, DC, 2014.
- [3] Konopacka, D.; Jesionkowska, K.; Kruczynska, D.; Stehr, R.; Schoorl, F.; Buehler, A.; Egger, S.; Codarin, S.; Hilaire, C.; Holler, I.; Guerra, W.; Liverani, A.; Donati, F.; Sansavini, S.; Martinelli, A.; Petiotj, C.; Carbó, J.; Echeverria, G.; Iglesias, I.; Bonany, J. Apple and Peach Consumption Habits Across European Countries. *Appetite* **2010**, 55, 478–483.
- [4] Boyer, J.; Liu, R.H. Apple Phytochemicals and Their Health Benefits. *Nutrition Journal* **2004**, 3, 5–19.
- [5] Eberhardt, M.V.; Lee, C.Y.; Liu, R.H. Antioxidant Activity of Fresh Apples. *Nature* **2000**, 405, 903–904.
- [6] Le-Marchand, L.; Murphy, S.P.; Hankin, J.H.; Wilkens, L.R.; Kolonel, L.N. Intake of Flavonoids and Lung Cancer. *Journal of the National Cancer Institute* **2000**, 92, 154–160.
- [7] Xing, N.; Chen, Y.; Mitchell, S.H.; Young, C.Y.F. Quercetin Inhibits the Expression and Function of the Androgen Receptor in LNCaP Prostate Cancer Cells. *Carcinogenesis* **2001**, 22, 409–414.
- [8] Gallus, S.; Talamini, R.; Giacosa, A.; Montella, M.; Ramazzotti, V.; Franceschi, S.; Negri, E.; Le Vecchia, C. Does An Apple a Day Keep the Oncologist Away? *Annals of Oncology* **2005**, 16, 1841–1844.

- [9] Butland, B.K.; Fehily, A.M.; Elwood, P.C. Diet, Lung Function, and Lung Function Decline in a Cohort of 2512 Middle Aged Men. *Thorax* **2000**, *55*, 102–108.
- [10] Lee, K.W.; Kim, Y.J.; Kim, D.; Lee, H.J.; Lee, C.Y. Major Phenolics in Apple and Their Contribution to the Total Antioxidant Capacity. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 6516–6520.
- [11] Tsao, R.; Yang, R.; Christopher, J.; Zhu, Y.; Zhu, H.H. Polyphenolic Profiles in Eight Apple Cultivars Using High-Performance-Liquid-Chromatography (HPLC). *Journal of Agricultural and Food Chemistry* **2003**, *51*, 6347–6353.
- [12] Balázs, A.; Tóth, M.; Blazics, B.; Héthelyi, E.; Szarka, S.; Ficsor, E.; Ficzek, G.; Lemberkovics, É.; Blázovics, A. Investigation of Dietary Important Components in Selected Red Fleshed Apples by GC–MS and LC–MS. *Fitoterapia* **2012**, *83*, 1356–1363.
- [13] Tsao, R.; Yang, R.; Xie, S.; Sockovie, E.; Khanizadeh, S. Which Polyphenolic Compounds Contribute to the Total Antioxidant Activities of Apple? *Journal of Agricultural and Food Chemistry* **2005**, *53*, 4989–4995.
- [14] Jaganath, I.B.; Crozier, A. *Plant Phenolics and Human Health: Biochemistry, Nutrition, and Pharmacology*; John Wiley & Sons, Inc., Hoboken, New Jersey. **2010**.
- [15] Robards, K.; Antolovich, M. Analytical Chemistry of Fruit Bioflavonoids. *Analyst* **1997**, *122*, 11–34.
- [16] Khanizadeh, S.; Tsao, R.; Rekika, D.; Yang, R.; Charles, M.T.; Rupasinghe, V.H.P. Polyphenol Composition and Total Antioxidant Capacity of Selected Apple Genotypes for Processing. *Journal of Food Composition and Analysis* **2008**, *21*, 396–401.
- [17] Łata, B.; Trąmpczyńska, A.; Paczeńska, J. Cultivar Variation in Apple Peel and Whole Fruit Phenolic Composition. *Scientia Horticulturae* **2009**, *121*, 176–181.
- [18] Wu, J.; Gao, H.; Zhao, L.; Liao, X.; Chen, F.; Wang, Z.; Hu, X. Chemical Compositional Characterization of Some Apple Cultivars. *Food Chemistry* **2007**, *103*, 88–93.
- [19] Wolfe, K.; Wu, X.; Liu, R.H. Antioxidant Activity of Apple Peels. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 609–614.
- [20] EU. DOOR, Database of Origin and Registration, European Commission. <http://ec.europa.eu/agriculture/quality/door> (accessed April 13, 2015).
- [21] Kim, D.O.; Seung, W.J.; Lee, C.Y. Antioxidant Capacity of Phenolic Phytochemicals from Various Cultivars of Plums. *Food Chemistry* **2003**, *81*, 321–326.
- [22] Singleton, V.L.; Rossi, J.A. Colorimetry of Total Phenolics with Phosphomolybdic–Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture* **1965**, *16*, 144–158.
- [23] Zhishen, J.; Mengcheng, T.; Jianming, W. The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals. *Food Chemistry* **1999**, *64*, 555–559.
- [24] Lee, J.; Durst, R.W.; Wrolstad, R.E. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *Journal of AOAC International* **2005**, *88*, 1269–1278.
- [25] Oliveira, A.; Gomes, M.H.; Alexandre, E.M.C.; Poças, F.; Almeida, D.P.F.; Pintado, M. Phytochemicals Preservation in Strawberry As Affected by pH Modulation. *Food Chemistry* **2015**, *170*, 74–83.
- [26] USDA. USDA National Nutrient Database for Standard Reference. Agricultural Research Service. National Agricultural Library. <http://ndb.nal.usda.gov> (accessed April 13, 2015).
- [27] Drogoudi, P.D.; Michailidis, Z.; Pantelidis, G. Peel and Flesh Antioxidant Content and Harvest Quality Characteristics of Seven Apple Cultivars. *Scientia Horticulturae* **2008**, *115*, 149–153.
- [28] Imeh, U.; Khokhar, S. Distribution of Conjugated and Free Phenols in Fruits: Antioxidant Activity and Cultivar Variations. *Journal of Agricultural and Food Chemistry* **2002**, *50*, 6301–6306.
- [29] Vrhovsek, U.; Rigo, A.; Tonon, D.; Mattivi, F. Quantitation of Polyphenols in Different Apple Varieties. *Journal of Agricultural and Food Chemistry* **2004**, *52*, 6532–6538.
- [30] Ramos, S. Effects of Dietary Flavonoids on Apoptotic Pathways Related to Cancer Chemoprevention. *Journal of Nutritional Biochemistry* **2007**, *18*, 427–442.
- [31] Downs, R.J.; Siegelman, H.W.; Butler, W.L.; Hendricks, S.B. Photoreceptive Pigments for Anthocyanin Synthesis in Apple Skin. *Nature* **1965**, *205*, 909–910.
- [32] Zamora-Ros, R.; Andres-Lacueva, A.; Lamuela-Raventós, R.M.; Berenguer, T.; Jakszyn, P.; Barricarte, A.; Ardanaz, E.; Amiano, P.; Dorronsoro, M.; Larrañaga, N.; Martínez, C.; Sánchez, M.J.; Navarro, C.; Chirlaque, M.D.; Tormo, M.J.; Quirós, J.R.; González, C.A. Estimation of Dietary Sources and Flavonoid Intake in a Spanish Adult Population (EPIC-Spain). *Journal of the American Dietetic Association* **2010**, *110*, 390–398.