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# Phenolic Compounds and Antioxidant Capacity of Brazilian Apples

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## Abstract

**Apple consumption is related to the prevention of chronic diseases due to the presence of phenolic acids and flavonoids that have antioxidant capacity. However, phenolic profile and antioxidant capacity can vary between varieties. The aim of this study was to analyze the total phenolic content (TPC) and antioxidant capacity (AC) of thirty-six apple varieties to establish a classification of these fruits. The TPC was analyzed according to the Folin-Ciocalteau method and AC was analyzed using the ferric reducing antioxidant power (FRAP) method. Hierarchical cluster analysis was used for the classification of the fruit. The TPC ranged from 456 to 1583 mg/kg of fresh fruit (catechin equivalents) and the AC ranged from 5606 to 23,719 mmol/kg (fresh fruit). The Pearson linear correlation showed significant differences in the association between TPC and AC (r: 0.79, $p < 0.001$). The apples were classified into the following five groups according to their AC: very high (8%), high (17%), medium (47%), low (11%) and very low (17%). The varieties classified in the group with very high AC were Carícia, Mollies and Imperatriz. The Gala varieties showed a great variability in AC, with values classified as high and low, while the Fuji, with less variability, was classified as low AC. The classification of apples based on TPC and AC using hierarchical cluster analysis was performed and the results can be of great value to geneticists, producers and consumers.**

## Keywords

***Malus* sp., FRAP, Phenols, Antioxidant Activity, Multivariate Analysis**

## 1. Introduction

Daily apple consumption is related to the reduction of the incidence of chronic non-communicable diseases

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(NCD), such as cardiovascular diseases and cancer [1]-[4], as well as some functional disorders associated with aging, such as diabetes and cholesterol [5], due to the presence of phytochemicals, especially flavonoids [6]. Apart from phenolic compounds, the presence of fibers [7], organic acids and phytosterols [8] in apple peel also contribute to the functional and nutritional value of the fruit. Vitamin C can be found in low concentrations [9], without a significant influence on the antioxidant capacity.

The ability to scavenge free radicals and pro-oxidant metals of phenolic compounds can explain a reduction in NCD. However, D'Archivio *et al.* [10] suggest that phenols act in the modulation of the activity of enzymes, in the interaction of receptors and signal transduction pathways.

The relationship between the concentration of total phenols, the phenolic profile, and the antioxidant capacity of apples from different countries has been demonstrated [11]-[13]. The epicarp (peel) has two to four times more phenolic compounds than the mesocarp (pulp), depending on the cultivar [13]-[15], which interferes in the antioxidant capacity of the fruit. The consumption of apple pulp can provide 48% - 78% of the antioxidant capacity of the fruit; however, if it is ingested with the peel, this value may increase to 79% to 89% [14]. Total phenolic content and phenolic profile are influenced by genetic factors, developmental stage and environmental conditions [15] [16], which directly affect the antioxidant capacity of apples.

Apples are the third most consumed fruit in Brazil [17], with a per capita consumption of 5.78 kg/cap/year in 2012, but in lower amounts than observed in several apple producing countries (10 kg/cap/year) [18]. China is the largest producer of apples, followed by the USA. Poland, Italy and France are important producers in the European Union [17]. Brazil is ranked in 9th place, with production of over 1,300,000 tons of apples in the 2012-2013 harvest [19]. Argentina and Chile produce a similar amount to Brazil and are the main competitors in terms of both the internal and external markets [18].

Brazilian apple production is exclusively composed of fruit for fresh consumption. There are many varieties and pollinating cultivars that have resulted from genetic work (hybridizations, clonal selections and mutations); however, more than 95% of the orchards are formed by Gala (55%) and Fuji (40%) varieties and their clones [20].

The recent increased in Brazilian apple production, which made high quality fruit available to consumers throughout the year, combined with nutritional fruit potential, was the motivation for this study. Therefore, the aim of this study was to evaluate and classify fresh apples produced in Brazil based on their total phenolic content and antioxidant capacity.

## 2. Materials and Methods

Thirty-six dessert apple varieties (commercial, pollinating and cultivars at an advanced stage of genetic improvement) from two consecutive harvests, at a ripening stage acceptable for consumption [21] (10 kg each), were used. These varieties represent most of the apples produced in Brazil. The samples, from the states of Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS), were supplied by small producers and the Agricultural Research and Rural Extension of Santa Catarina (EPAGRI/SC), Agricola Fraiburgo (Fraiburgo/SC) and Boutin Fruticultura (Porto Amazonas/PR). The reagents used were of analytical grade.

### 2.1. Extraction of Phenolic Compounds

The apples were selected in order to exclude those with phytopathological defects or mechanical damage. The whole fruits were then weighed and crushed in a multiprocessor (METVISA, Tipo MPA, Brazil). Immediately, L-cysteine was added (2 mmol, C-7880, Sigma-Aldrich, Germany) to the crushed fruit to avoid oxidation [14]. To extract the phenolic compounds from the crushed apple, a solution composed of 70% ethanol: water: 3% formic acid (80:20:1) in the proportion 1:1 (w/v) was prepared, as described by McGhie *et al.* [22]. The mixture (apple and solution) was homogenized using a mixer (Black & Decker, SB40 model, Brazil) and kept at −18˚C for 18 - 24 hours. The samples were then centrifuged (CELM, COMBATE, 3548 Series, Brazil) for 20 minutes at 3400 rpm and the supernatant (phenol extract) was separated for analysis [14].

### 2.2. Total Phenolic Content (TPC)

Colorimetric analysis using Folin Ciocalteau, as described by Singleton and Rossi [23], was performed. A control was performed with enzyme inhibitor (L-cysteine). The results were expressed in mg/kg of total phenols using catechin (Sigma-Aldrich Co., C-1251, Germany) as standard.

### 2.3. Antioxidant Capacity (AC)

The spectrophotometric method of "ferric reducing antioxidant power" (FRAP) was used, as described by Pulido *et al.* [24] and Benzie and Strain [25]. The absorbance was measured at 593 nm. After the first measure (L1), which was performed within 5 seconds, the absorbance was monitored for 6 minutes (L6) every 15 seconds. The result was expressed as mmol of iron reduction/antioxidant power (FRAP) per kilogram of sample. The absorbance values obtained were calculated by differences between L1 and L6 and compared to a calibration curve of 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (0.1 to 1.0 mmol/L; $R^2 = 0.99$) (TROLOX, Flucka Chemicals Suisse, Switzerland, 56,510).

### 2.4. Statistical Analysis

The data were presented as mean and coefficient of variation (CV). Prior to the application of Hierarchical cluster analysis (HCA), all the variables were autoscaled. The similarities of the samples were calculated based on the square of the Euclidean distance, and the incremental method of hierarchical clustering was used to establish the clusters. Hierarchical cluster analysis (HCA) was performed using Pirouette® v.4.0 (Infometrix, Bothell, WA, USA) software. Levene's test was conducted to check the homogeneity of variances; one-way ANOVA and Tukey's test were applied to evaluate the differences between the clusters. All other statistical analyses were performed using STATISTICA 7.1 software (Stat-Soft Inc, Tulsa, OK, USA).

## 3. Results

The phenolic compound content of the apples (**Table 1**) ranged from 456 to 1583 mg/kg of fresh fruit (catechin equivalents). Gala and Fuji are the major varieties produced in Brazil and the phenolic content in these fruits is on average 1082 and 1144 mg/kg, respectively. The sample of IAPAR 180 had the lowest phenol content, while the Daiane variety (developed from a genetic cross of Gala and Princesa apples) had values that reached 2206 mg/kg.

High coefficients of variation (CV) were found for the TPC analysis in some cultivars, such as M-2/01 (55.08%), Daiane (51.68%), Joaquina (43.42%), Gala (42.23 and 20.85% from Paraná and Santa Catarina State, respectively) and Golden Delicious (19.96%).

The antioxidant capacity of the samples ranged from 5606 to 23,719 mmol/kg of fresh fruit. The Carícia variety, produced in Paraná showed the highest value. The Gala variety (PR) has the highest coefficient of variation for this analysis (**Table 1**). The results of the Pearson linear correlation analysis showed a significant association between TPC and AC (r: 0.79; $p < 0.001$).

Hierarchical cluster analysis (HCA) was used to evaluate the similarity of the samples and to suggest a classification of Brazilian apples according to TPC and AC (**Figure 1**). Five clusters were suggested and the fruits were divided into three classes of AC: high (Clusters 1 and 2), medium (Cluster 3) and low (Clusters 4 and 5).

As can be seen in the dendrogram shown in **Figure 1**, among the 36 samples of Brazilian apples that were analyzed, 25% were classified as apples with high AC (Clusters 1 and 2). Of these, the Carícia (PR), Mollies (SC) and Empress (RS) varieties (Cluster 1) belonged to the very high AC group (**Table 2**). The M6/00, Eva (SC), M11/01, M12/00, M13 and IAPAR 180/00 varieties had the lowest TPC and levels of AC (Cluster 5) and were part of the very low AC group (**Table 2**).

Of the varieties that are most produced in Brazil, the Gala variety from Rio Grande do Sul was among the samples of higher AC and was classified in the group with high AC (Cluster 2). The Golden Delicious (Cluster 3) showed medium AC and the Fuji was classified in the low AC group (Cluster 4).

## 4. Discussion

The TPC in apples varies considerably depending on the cultivar and the different parts of the fruit, as well as factors related to the location of cultivation and harvest, and harvest time [13] [14]. Carbone *et al.* [11] detected TPC of 1410 mg/kg (fresh fruit) in Italian apple cultivars (Fuji, Braeburn and Golden Delicious), which were similar to those found in this study. However, the lowest value found by the same authors was 920 mg/kg, which was much higher than the values in this study (456 mg/kg). The selection of cultivars with low astringency and bitterness, due to consumer preference, may explain the low content that was observed.

**Table 1.** Average of total phenolic content (TPC) and antioxidant capacity (AC) in fresh weight of 36 cultivars of apples produced in southern Brazil.

| Varieties | Origin[a] | NR[b] | Analysis | | | |
|---|---|---|---|---|---|---|
| | | | CFT (mg/kg) | CV[c] (%) | CA (mmol/kg) | CV[c] (%) |
| Carícia | PR | 3 | 1583 | 2.69 | 23,719 | 0.60 |
| Mollies | SC | 3 | 1121 | 0.61 | 21,906 | 0.99 |
| Imperatriz | RS | 3 | 1298 | 2.81 | 21,102 | 0.97 |
| Catarina | SC | 6 | 1391 | 5.09 | 19,113 | 7.74 |
| Imperial Gala | SC | 3 | 1123 | 2.31 | 18,907 | 7.49 |
| Fuji Suprema | SC | 3 | 1243 | 0.23 | 18,844 | 11.13 |
| Mollies | RS | 3 | 1430 | 1.87 | 17,814 | 3.77 |
| Gala colorida | SC | 3 | 1118 | 1.06 | 17,807 | 3.35 |
| Gala | RS | 3 | 1245 | 2.25 | 17,257 | 2.14 |
| Sansa | SC | 3 | 1109 | 1.74 | 16,874 | 6.38 |
| Golden delicious | SC | 9 | 1055 | 19.96 | 16,445 | 28.69 |
| P-23[d] | SC | 3 | 1046 | 1.60 | 15,848 | 0.82 |
| Royal Gala | SC | 3 | 1107 | 0.91 | 15,635 | 3.98 |
| Princesa | PR | 3 | 811 | 1.78 | 15,496 | 3.29 |
| M-13-91[d] | SC | 9 | 985 | 9.50 | 15,259 | 22.60 |
| M-2/01[d] | SC | 6 | 1043 | 55.08 | 15,055 | 2.08 |
| Cherry Gala | SC | 3 | 965 | 4.26 | 14,894 | 3.17 |
| Daiane | SC | 9 | 1382 | 51.68 | 14,478 | 24.24 |
| Gala | SC | 15 | 1035 | 20.85 | 14,327 | 25.82 |
| Akifu | SC | 3 | 933 | 0.87 | 14,251 | 2.74 |
| M-8/01[d] | SC | 3 | 1352 | 3.08 | 14,073 | 2.90 |
| MRC-11/95[d] | SC | 6 | 1114 | 8.20 | 13,932 | 5.82 |
| Duquesa | SC | 3 | 792 | 3.50 | 13,850 | 4.01 |
| Joaquina | SC | 9 | 1368 | 43.42 | 13,802 | 8.98 |
| Braeburne | SC | 3 | 726 | 9.90 | 13,793 | 1.83 |
| Royal Gala | PR | 6 | 1187 | 3.57 | 13,671 | 4.14 |
| M-11/00[d] | SC | 9 | 888 | 14.83 | 13,117 | 23.07 |
| Fuji | SC | 12 | 1144 | 9.49 | 12,957 | 12.38 |
| Gala | PR | 6 | 966 | 42.23 | 12,340 | 47.58 |
| Condessa | SC | 3 | 602 | 1.00 | 11,431 | 4.09 |
| M-6/00[d] | SC | 6 | 661 | 13.97 | 9810 | 15.79 |
| Eva | SC | 3 | 598 | 3.51 | 8811 | 5.14 |
| M-11/01 | SC | 3 | 736 | 3.00 | 8491 | 0.59 |
| M-12/00 | SC | 6 | 616 | 12.73 | 8364 | 35.47 |
| Iapar 180 | SC | 3 | 456 | 1.32 | 7157 | 4.01 |
| M-13/00[d] | SC | 6 | 594 | 13.69 | 5606 | 40.51 |

Note: [a]PR: Paraná State. SC: Santa Catarina State, RS: Rio Grande do Sul State; [b]number of repetitions of each batch (10 kg); [c]coefficient of variation; [d]apples under evaluation for development of new cultivars.

**Table 2.** Mean values of total phenolic compounds (TPC, mg/kg) and antioxidant capacity (AC, mmol/kg), and classification of varieties of Brazilian apples based on AC.

| Parameters | Clusters | | | | | PSD | P Value[b] | P Value[c] |
|---|---|---|---|---|---|---|---|---|
| | C1 | C2 | C3 | C4 | C5 | | | |
| FT | 1333.86[a] | 1258.41[a] | 1059.40[ab] | 900.00[bc] | 610.25[c] | 277.87 | 0.50 | <0.001 |
| CA | 22242.48[a] | 18290.17[b] | 14805.02[c] | 12510.02[d] | 8039.83[e] | 4029.10 | 0.55 | <0.001 |
| N[(*)] | 3 (8%) | 6 (17%) | 17 (47%) | 4 (11%) | 6 (17%) | - | - | - |
| Classes | Very High | High | Medium | Low | Very low | - | - | - |

Note: [(*)]N = number of varieties. Columns with dashes (-) = data not calculated. Different letters in the same line represent significant difference ($p < 0.05$). [a]PSD: pooled standard deviation; [b] Probability values obtained by Levene's test for homogeneity of variances; [c]Probability values obtained by one-way ANOVA.

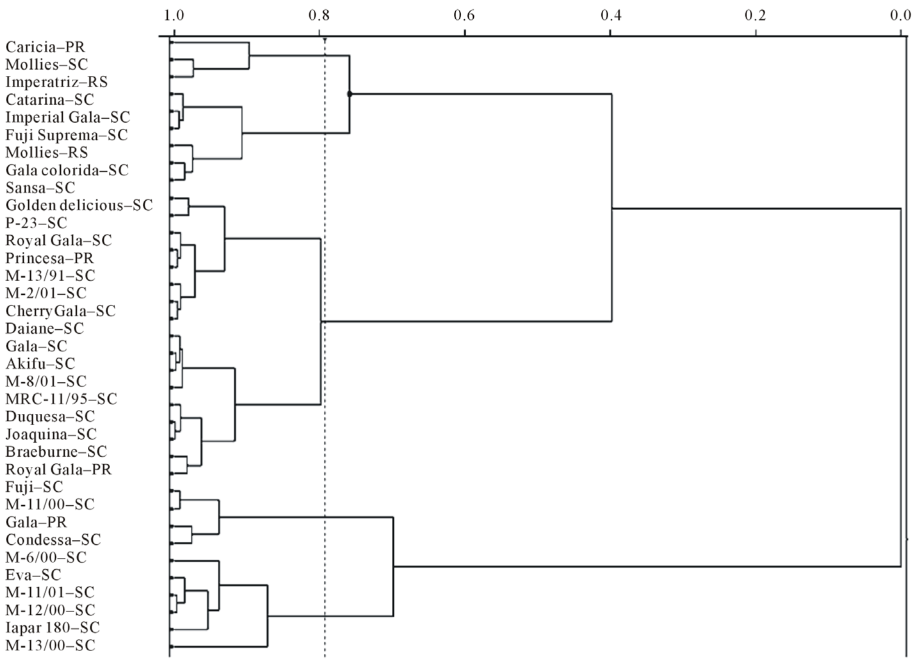


**Figure 1.** Dendrogram for Brazilian apple cultivars obtained from hierarchical cluster analysis.

Studies have shown a relationship between the intensity of the red color in the epicarp (skin) of the fruit and phenolic content, particularly in relation to anthocyanins [26] [27]. The Gala variety has no red coloration throughout the epicarp (peel), Fuji has a faded color, while the Supreme Fuji and Daiane varieties have epicarps with an intense red color [28]. The higher levels of pigmentation may be related to variations found in TPC levels in these apple cultivars.

Variations in TPC within the same cultivar, grown in the same state, can be justified by climatic variability [16] [29]. This could explain the high coefficients of variation attributed to the M-2/01, Joaquina, Gala and Golden Delicious cultivars, all of which were from Santa Catarina (**Table 1**).

The Gala (PR) and Daiane varieties also had high coefficients of variation (**Table 1**), which may be explained by differences in planting areas, crops and harvest seasons [30]. Two samples of the Gala (PR) variety were analyzed from the city of Porto Amazonas and they had an average TPC of 595 mg/kg, while a sample coming from the city of Lapa (40 km away) had nearly twice as much phenolic content (1337 mg/kg).

The results of this study are consistent with several previous studies which reported that apples may have elevated AC, and that this varies greatly among cultivars [11] [13] [15] [31]-[35]. Khanizadeh [13] found AC values for the Canadian Gala cultivar that ranged between 440 and 4.301 µg of ascorbic acid equivalent per gram of fresh fruit, in the peel and pulp, respectively. According to Wojdylo *et al*. [33], the difference in content between TPC in cultivars explains the variation in the observed AC, which could also be seen in this study, where the TPC concentration ranged between 456 and 1583 mg/kg fresh fruit (**Table 1**). Furthermore, McGhie *et al.* [22] reported that difference in AC occurs due to variations in phenolic profile and not only due to the TPC.

According to Noakes and Roupas [36], the evaluation of the AC of natural products can be affected by many factors, and thus the development of a ranking using AC in apples is highly dependent on the method used and the phenolic composition of the fruit; it is difficult to compare values when using different methods and standards. Zheng *et al.* [37] found AC levels of 16.75 mmol Trolox equivalent per gram of fresh fruit in Fuji apples from Korea. Fu *et al.* [12] analyzed the AC of four cultivars of Chinese apples and the average value was 7.8 micromol Fe (II)/g of fresh sample.

A high coefficient of variation was observed in the analysis of AC, as can be seen in **Table 1**. This can be explained by the variability observed in the TPC of some cultivars such as Gala (PR).

The results obtained from HCA (**Figure 1**) were similar to those reported by Lee and Smith [38] with respect to the percentage of apples classified as having high AC. In that study, the authors analyzed and classified 24 samples of apples grown in New York, USA and 33.3% belonged to the group with high AC; in our study, 25% of the samples were classified in this group. In contrast, as can be seen in **Table 2**, about 17% of the fruits that were examined were classified in the group with very low AC.

The expression “an apple a day keeps the doctor away”, which was created in the nineteenth century in Wales, highlights the enormous powers of this fruit to ensure improvements in health and to prevent diseases. Studies have indicated that the consumption of apples may be enough to provide antioxidants in our organism and thus obtain the desired preventive effect [39]-[43]. However, to achieve these beneficial effects it is necessary to consume an apple with high AC or, if the fruit has a low AC (**Table 2**), it would require the consumption of two point three (2.3) fruits to obtain the same result.

Thus, an apple rating system based on TPC and AC can be useful for the following: to visualize the variability that a cultivar may present or not; to identify cultivars with functional potential; to valorize cultivars and influence production; to provide information about the breeding of new cultivars; and to generate information for the benefit of consumers.

## 5. Conclusions

The apple cultivars that were analyzed showed differences in total phenolic content (TPC) and antioxidant capacity (AC). The Daiane cultivar, with higher (average) levels, presented three times more polyphenols than a cultivar with lower TPC (IAPAR 180).

The apples were classified into five groups according to their AC, very high (8%), high (17%), medium (47%), low (11%) and very low (17%). The varieties classified in the group with very high AC were Carícia (PR), Mollies (SC) and Imperatriz (RS). The Gala varieties showed great variability in AC, with values classified as high and low, while the Fuji variety showed less variability and was classified as low AC.

Among the cultivars that were most produced in Brazil, the Gala from Rio Grande do Sul demonstrated the highest AC and it was classified in the group with high AC (Cluster 2), while those produced in Santa Catarina and Paraná were classified in the medium AC group (Cluster 3) and low AC (Cluster 4), respectively. The Fuji was also classified in the low AC group (Cluster 4).

The results indicated that the consumption of antioxidant polyphenols from apples can be increased simply by the appropriate choice of cultivar. Thus, this information can be useful to both consumers, who are interested in knowing which variety can have a greater beneficial effect on their health, as well as producers, who can use this information for production and marketing purposes.

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