Physicochemical properties and antioxidant activity of two varieties of apple cultivated in different areas in Morocco

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Abstract: This study aimed to assess the effect of climate on the organoleptic qualities and physiochemical properties of two varieties of apple (Golden delicious (GD) and Red delicious (RD)) collected from different regions in Morocco. These two varieties of apple were examined for their bioactive compounds composition and antioxidant activity (in juices and different extracts). For physiochemical parameters, the highest acidity was observed in Golden delicious collected from station 1, which was the juiciest sample. Bioactive substances content was dependent on variety and station, while the highest polyphenol content was observed in Golden delicious collected from station 2 (135.41±6.66 mg GAE/100 mL of juice) and most top flavonoid content was observed in Red delicious collected from station 1 (7.43±0.13 mg QE/100 mL of juice). Furthermore, Red delicious collected from station 2 was the most effective in chelating the radical DPPH (IC50% = 0.92±0.01 µL of juice), and Red delicious collected from station 1 has the highest total antioxidant activity (2.47±0.02 mg AAE/100 mL of juice). The present work showed that the significant diversity in the different studied parameters of the apple cultivars was closely linked with the characteristics of the station, such as geography and climate.

Keywords: Golden delicious; red delicious; apple juice; antioxidant activity; polyphenols; flavonoids.

1. Introduction

The importance of fruits and their byproducts for human health cannot be overstated. Fruits are considered an essential part of the daily diet and are on high demand because they ensure a balanced diet 1. Fruits are an inexhaustible source of bioactive constituents 2,3. Apple serves as a source of different dietary phytonutrients, which possess many pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic 3-5.

The daily consumption of apples can play a role in decreasing the risk of different chronic diseases such as cardiovascular disease, cancer and diabetes 7-9.

Apples are one of the best sources of bioactive compounds 10. Several studies correlated the bioactive compounds with health-protective activities 2,4-6,11. In the last decades, bioactive compounds earned remarkable attention for their biological activities 12.

Apples are comprised of several biologically active substances, such as carbohydrates, fiber, vitamins, organic fruit acids, hydroxycinnamic acids, flavonols and anthocyanins 13.

However, geographical conditions influence the content of some of these bioactive substances content in fruits. In Morocco, the apple crops constitute an important source of income for the Moroccan populations in mountainous areas. Geographical localization of Morocco offers a complete range of Mediterranean bio-climates favoring the development and fruiting of different apple varieties 14. The apple tree currently occupies an area of about 63000 hectares and ranks as the second among the cultivated rosacea, after the almond tree, according to statistical forecasts reported by Sellika 15. Products based on apple are numerous and diversified, and they include juice and vinegar. The transformation of apples, unfortunately, remains weak or even absent in Morocco 16.

This research aimed to assess the proximate physicochemical properties, the antioxidant activity of two varieties of apple cultivated in different areas in Morocco as well as the possible impact of pedoclimatic conditions on bioactive components composition of fruits.
2. Materials and methods

2.1. Plant material and harvest location
The plant material was composed of apple fruits of two varieties, Golden delicious and Red delicious; there were collected in October 2019 from different regions in Morocco (station 1: Imilchil, station 2: Ait ayach and station 3: Emmouzzer). The climatic and geolocalisation data are presented in Table 1.

Table 1. Characteristics of sampling sites.

| Climate data          | Station 1                  | Station 2                  | Station 3                  |
|-----------------------|----------------------------|----------------------------|----------------------------|
| Latitude              | 32°3'8.594N                | 32° 41'23.124N             | 33°44'0.24 N               |
| Longitude             | 5°46'9.105W                | 4°54'29.33W                | 5°0'37.8W                  |
| Altitude              | 2138                       | 1516                       | 1317                       |
| Rainfall mm/year      | 474                        | 263                        | 612                        |
| Annual temperature °C | 11                         | 15.20                      | 13.5                       |
| Bioclimatic stage     | humid                      | Semi-arid                  | Sub-humid                  |

2.2. Preparation of extracts and juices
The extraction process was performed with two solvents as follows: ethanol 70%, methanol 80% with sonication for 30 min. The solid to liquid report was 1:10. The obtained extracts were filtered through filter paper and then were analyzed.

For the preparation of juice, the fruits were washed in distilled water and separately blended using a Moulinex blender. The juices were filtered through filter paper and conserved before the analysis. Different samples of juice were named as follows: S1GD: Station 1 Golden delicious, S1RD: station 1 Red delicious, S2GD: station 2 Golden delicious, S2RD: station 2 Red delicious, S3GD: Station 3 Golden delicious, S3RD: Station 3 Red delicious.

2.3. Physicochemical properties
The pH of juices of different apple varieties was measured by a pH meter (OHAUS ST2100 F). Electric conductivity was measured using a conductivity meter (CD20 Conductivity meter) and was expressed as microsiemens per centimeter (µS/cm) \(^{16}\). The total acidity was determined titrimetrically according to the French standard \(^{18}\). Brix and density were measured using a refractometer.

2.4. Phytochemical screening
2.4.1. Total polyphenols and flavonoids contents
The total phenolic content (TPC) was estimated according to the colorimetric method as reported by Singleton and Rossi \(^{19}\), using Folin-Ciocalteau reagent, with some modifications. Briefly, 200 µL of juice or extract was mixed with 1 mL of Folin-Ciocalteau (0.2N) reagent and 800 µL of sodium carbonate solution. After 2 hours of incubation, the absorbance of the mixture was measured at 760 nm. The results were expressed as mg of gallic acid equivalent per g of fruit and mg of gallic acid equivalent per 100 mL for the juice. The total flavonoids (TFC) content was performed using the aluminum trichloride’s method described by Kong et al. \(^{20}\). Briefly, 200 µL of juice or extract was mixed with 300 µL sodium nitrite, 300 µL of AlCl\(_3\) and 600 µL of sodium acetate. Results were expressed as mg of quercetin equivalent per g for fruit and as mg of quercetin per 100 mL for the juice. Tests were carried out in triplicates.

2.5. Antioxidant activity
2.5.1. DPPH radical scavenging assay
The DPPH radical scavenging assay was used to examine the antioxidant activity of different extracts and juices. The radical scavenging activity was measured as previously described by Hogg and Russell \(^{21}\). The antioxidant capacity of the various extracts and juices studied were interpreted from their IC50. The value of IC50 was expressed in mg/mL for different extracts and µL/mL for juice.

2.5.2. Total antioxidant capacity
To evaluate the antioxidant capacity (TAC) of the extracts and juices, the phosphomolybdenum method was used as described by Zengin et al. \(^{22}\). The antioxidant capacity of different extracts and juices was evaluated as ascorbic acid equivalents (mg AAE/100g of fruit and mg AAE/100 mL of juice, respectively).

2.6. Statistical analysis
Statistical analysis was carried out by one-way ANOVA through the Past 3 program to determine the significance (P<0.05). Correlations between the characterizing parameters of samples were achieved by the Pearson correlation coefficient (r) at a significance level of 99% (P<0.05). The results were also subjected to a multivariate analysis (principal component analysis).

3. Results and Discussion
Physicochemical analyses of juices are summarized in Table 2. The pH which was recorded in different samples ranged between 3.31±0.01 and 4.05±0.01; it was slightly acidic, especially in S1GD. Electrical conductivity values ranged between 221.67±2.51 and 241.33±6.42 µS/cm for S2GD and S1GD.
respectively. Titratable acidity (TA) content (expressed as g of malic acid per 100 mL of juice) varied from 0.48±0.133 (S2GD) to 1.82±0.13 (S1GD). Concerning the juiciness, the S1GD sample had the highest juiciness value with 67.5 mL/100 g, whilst, the lowest juiciness was established in sample S3RD. The results of this study indicate that the density of all samples ranged between 1.1257 (S3GD) and 1.1297 (S1RD). The values of °Brix varies within 16.2 (S3GD) and 16.65 (S1RD).

The obtained results for pH are comparable to those documented for some fruits such as orange, kiwi fruit and apple 23, which ranged from 3.23 to 4.22. Similar findings have been published for the apple in Poland, Tunisia and China respectively 24–26. The results of TA from this study were higher than those documented by Boudabous et al. 24 but similar to those reported previous studies 23,26. Acidity is one of the elements used to assess fruits quality 27, and it gives the sour taste due to the presence of malic acid 28. The results of juiciness from this study were in agreement with those reported by Piagentini and Pirovani (2017) 28. The release of juice and moisture content has an impact on juiciness 28.

The results indicate that the climate of each station impacts the physicochemical properties of apple varieties studied.

### Table 2. Physicochemical parameters of juice samples.

| Station | Apple variety | pH     | Electrical conductivity µS/cm | Titratable acidity % | Juiciness mL/100 g fw | Density fw | °Brix |
|---------|---------------|--------|-------------------------------|----------------------|------------------------|------------|-------|
| S1      | GD            | 3.31±0.01 | 241.33±6.42                  | 1.82±0.13            | 67.5                   | 1.1271     | 16.36 |
|         | RD            | 4.01±0.015 | 230.66±6.43                  | 0.86±0.145           | 61.76                  | 1.1297     | 16.65 |
| S2      | GD            | 4.03±0.02  | 221.67±2.51                  | 0.48±0.133           | 58.82                  | 1.1269     | 16.33 |
|         | RD            | 4.05±0.01  | 225±6.55                     | 0.67±0.136           | 66.37                  | 1.1283     | 16.49 |
| S3      | GD            | 4.1±0.01   | 223.34±9.01                  | 0.68±0.144           | 60                    | 1.1257     | 16.2  |
|         | RD            | 3.86±0.015 | 237.35±1.52                  | 0.60±0.04            | 53                    | 1.1266     | 16.3  |

S1GD: Station 1 Golden delicious, S1RD: Station 1 Red delicious.
S2GD: Station 2 Golden delicious, S2RD: Station 2 Red delicious.
S3GD: Station 3 Golden delicious, S3RD: Station 3 Red delicious.

The results of the bioactive compounds, namely, polyphenolic compounds and flavonoids, are illustrated in Table 3. The values vary according to varieties and the stations from which the varieties were collected. The highest content of phenolic compounds was 135.41±6.66 and 115.91±3.16 mg GAE/100 mL of juice established in sample S2GD for Golden delicious and S1RD for Red delicious respectively. The results for TPC in various sample extracts from different stations are presented in Table 3. Their quantity varied within the range of 7.16±0.72 to 3.5±0.3 mg AGE/ g fw for the Golden delicious and 7.89±0.28 to 3.88±0.02 mg AGE/ g fw for the Red delicious. The obtained results are in agreement with previous reports 29–31. The differences among varieties regarding the TPC could be due to the variation in geographic origin, climatic conditions, technical itineraries, maturity stages, conditions of storage and influence of cultivar 30,32,33.

Total flavonoids content of our analyzed juices varies between 6.76±0.02 mg QE/100 mL and 3.10±0.02 mg QE/100 mL from S1GD and S3GD, respectively. In analyzing both varieties, the Golden delicious of station 2 and Red delicious of station 1 accumulated the highest content of total phenolic substances. In addition, both varieties of station 1 accumulated the highest content of flavonoids. The sharp decrease can explain the fluctuation of flavonoids content in flavonoids during the fruit development 34. Other factors such as agricultural practices or storage condition can also change the bioactive components of apple and therefore their antioxidant potential.

The total antioxidant activity and the ability of the studied samples to scavenge free radicals DPPH were commonly used to determine the antioxidant potential of various samples.

Total antioxidant capacity of studied samples varied from 2.47±0.02 mg QE/100 mL to 1.93±0.30 mg QE/100 mL of juice. The sample S1RD has the highest value of total antioxidant capacity, while the sample S1GD has the lowest value. Concerning the different extracts, the highest antioxidant capacity was registered in S2RD ethanol extract, with a value of 7.28±0.28 mg GAE/ g fw. While the sample S3GD methanol extract has the lowest antioxidant capacity (1.13±0.07 mg GAE/g fw). The results obtained from this study are lower than those obtained by Drogoudi et al. 35.

Regarding free radical scavenging activity (DPPH) of different samples, on the one hand, The IC50 DPPH of juices varies within 1.9±0.11 µL (S3RD) and 0.92±0.01 µL (S2RD). On the other hand, the
IC50% DPPH of different extracts varied from 2.46±0.05 mg (S3RDEE) to 0.75±0.4 mg (S1GDEE). These results indicate that the different samples have potential antioxidant activity. The different climate conditions could explain the difference in the antioxidant activity from one station to another. Apple trees adopted to the adverse pedoclimatic conditions by producing secondary metabolites, which involved in their protection during its development. In addition, bioactive compounds content of apples depends on the cultivars, storage conditions, and stage of maturity of fruits.

Table 3. Total phenolic content, total flavonoids content and antioxidant activity of different juices and extracts.

| Station | Sample | TFC mg GAE/100 mL for juice | TFC mg QE/100 mL for extract | TAC mg AAE/100 mL for juice | DPPH IC50% |
|---------|--------|-----------------------------|-----------------------------|-----------------------------|------------|
|         |        | mg GAE/g for extract        | mg QE/g for extract         | mg AAE/g for extract        |            |
| S1      | GD     | Juice 81.16±4.41             | 6.76±0.02                   | 1.93±0.306                  | 1.46±0.19  |
|         |        | EE 7.16±0.72                 | 1.44±0.23                   | 6.01±0.555                  | 0.75±0.43  |
|         |        | ME 7.6±0.41                  | 0.5±0.22                    | 3.6±0.517                   | 2.43±0.97  |
|         |        | Juice 115.91±3.16            | 7.43±0.13                   | 2.47±0.026                  | 1.82±0.02  |
|         |        | EE 5.15±1.12                 | 1.24±0.46                   | 5.42±0.613                  | 1.30±0.01  |
|         |        | ME 7.01±0.72                 | 7.03±0.71                   | 2.68±0.210                  | 1.23±0.11  |
| S2      | GD     | Juice 135.41±6.66            | 5.07±0.09                   | 2.06±0.090                  | 1.13±0.22  |
|         |        | EE 3.5±0.30                  | 0.55±0.23                   | 3.12±0.306                  | 1.99±0.11  |
|         |        | ME 3.82±0.45                 | 2.29±0.17                   | 2.29±0.249                  | 2.04±0.50  |
|         |        | Juice 107.34±4.08            | 3.91±0.04                   | 2.36±0.067                  | 0.92±0.01  |
|         |        | EE 7.89±0.28                 | 0.43±0.11                   | 7.28±0.280                  | 0.99±0.08  |
|         |        | ME 7.61±0.57                 | 1.17±0.90                   | 2.98±0.090                  | 1.77±0.05  |
| S3      | GD     | Juice 98.5±7.41              | 3.10±0.02                   | 2.37±0.028                  | 1.22±0.07  |
|         |        | EE 4.53±0.24                 | 0.30±0.02                   | 5.46±0.190                  | 2.17±0.14  |
|         |        | ME 5.17±0.2                  | 1.48±0.23                   | 1.13±0.070                  | 1.81±0.09  |
|         |        | Juice 94.91±3.66             | 4.91±0.11                   | 2.11±0.009                  | 1.9±0.11   |
|         |        | EE 3.88±0.02                 | 0.55±0.20                   | 5.63±0.081                  | 2.46±0.05  |
|         |        | ME 5.43±0.08                 | 3.56±0.46                   | 2.06±0.0503                 | 1.6±0.80   |

EE: Ethanol extract; ME: Methanol extract
TPC: Total Phenolic Content; TFC: Total Flavonoids Content; TAC: Total Antioxidant Capacity.

To better distinguish among the different samples studied in the present work, the principal component PCA was performed as a tool to explore the links among variables and similarities between samples studied. Two principal components were extracted in the PCA model of all samples analyzed (Figure 1). The two primary components (PCs) explained an accumulative variance of 71.875%.
Figure 1. Projection of different samples studied and variable on the factorial plane formed by the first two principal components

The first component explained 42.467% and represented in its positive part: S1GD, S2RD and S3RD, while the S2RD, S2GD and S3GD were dominating in the negative part.

The second principal component explained 29.408% of the given results and represented mainly the S1RD and S2RD in the positive part, while the S1GD, S2GD, S3 GD and S3RD in the negative part. Good discrimination was made among the cultivars under investigation, which were discriminated by the second component. The samples S1RD and S2RD were in the positive part of component 2, and S2GD, S3GD, S3RD, S1GD were in the negative part of the same component. Their homogeneity characterizes the S1RD and S2RD in term of phenolic compounds, which implicated a positive correlation with the total antioxidant activity and a negative correlation with IC50%DPPH. The S1GD was distant from all the other samples studied as a result of its high pH, electrical conductivity, titratable acidity and juiciness. According to the results obtained from PCA, it can be inferred that the distribution of different varieties studied correlated with the station and the variety.

A positive correlation was found between total phenolic compounds and overall antioxidant activity in juice and negatively correlated with IC50%DPPH ($r=0.26299$ and $-0.27477$, respectively) (Table 4).

The bioactive compounds have a remarkable contribution in the antioxidant activity of different varieties of apple as previously reported by previous studies 28,35.

Table 4. Pearson correlation coefficients between phytochemical parameters and antioxidant activities of different juices.

|       | pH          | EC          | TA          | Juiciness   | °Brix       | Density     | TPC         | TFC         | TAC         | IC50%DPPH   |
|-------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| pH    | 0.03365     | 0.009170    | 0.44083     | 0.8792      | 0.86349     | 0.14447     | 0.2807      | 0.09413     | 0.61654     |
| EC    | -0.84622    | 0.10216     | 0.90523     | 0.8979      | 0.9149      | 0.09098     | 0.2244      | 0.30824     | 0.14665     |
| TA    | -0.92076    | 0.72624     | 0.17193     | 0.8750      | 0.89435     | 0.14084     | 0.2521      | 0.35218     | 0.76086     |
| Juiciness | -39302   | 0.06326     | 0.63904     | 0.4638      | 0.47135     | 0.66292     | 0.6757      | 0.96273     | 0.32156     |
| °Brix | 0.08065     | 0.06816     | 0.08347     | 0.37501     | 0.00248     | 0.59697     | 0.1719      | 0.35241     | 0.69148     |
| Density| 0.09126     | 0.05679     | 0.070547    | 0.36921     | 0.9998      | 0.57812     | 0.1772      | 0.35104     | 0.70117     |
| TPC   | 0.67111     | -0.7424     | -0.67554    | -0.22871    | 0.2756      | 0.28933     | -0.0209     | 0.9685      | 0.61461     | 0.59821     |
| TFC   | -0.52879    | 0.58311     | 0.55579     | 0.21968     | 0.6390      | 0.63901     | -0.2087     | 0.71452     | 0.21299     | 0.93433     |
| TAA   | 0.73777     | -0.5038     | -0.4655     | 0.02485     | 0.4653      | 0.46647     | 0.26299     | -0.1927     | -0.04381    |             |
| IC50%DPPH | -0.26161 | 0.66847     | 0.16081     | -0.49199    | 0.2087      | 0.20197     | -0.27477    | 0.5948      | 0.6821      |             |
3. Conclusion

Physicochemical parameters and antioxidant activity of two apple varieties collected in different areas in Morocco are compared. The content of bioactive compounds depends strictly on the variety of apple. The results revealed that Golden delicious of station 2 (S2GD) was the richest in phenolic content among all the other samples studied. Also, geographical apple crops location had a remarkable effect on the content of bioactive compounds of apple fruits. We conclude that Golden delicious and Red delicious of mountainous regions are potential candidates to be inexhaustible sources of flavonoids and phenolic compounds. These apple varieties can be a basic product for the manufacturing of many by-products with nutritional values.

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