Phenolic composition and total antioxidant capacity of the fresh and commercial Tunisian apple juice

Hédi Hammouda 1,2*, Mohamed Journi 1, Malika Trabelsi-Ayadi 1 and Jamila-Kalthoum Chérif 1,2

1 Laboratoire d’Application de la Chimie aux Ressources Naturelles et à l’Environnement (LACReSNE), Faculté des Sciences de Bizerte, 7021 Zarzouna-Bizerte, Tunisie.
2 Institut Préparatoire aux Études d’Ingénieurs de Tunis (IPEIT), Monfleury 1008 Tunis–Tunisie.

ABSTRACT

Six varieties of the fresh Tunisian apple juice and three varieties of the commercial juice were studied on the basis of their detailed polyphenolic profile. The separation and identification of the compounds were carried out by High performance Liquid Chromatography on Reversed Phase (RP-HPLC). Thiolysis coupled to RP-HPLC was used to determine the nature, the proportion of the constitutive units and the concentration of procyanidins. Moreover, it gave access to the calculation of the average degree of polymerization (DPn flavan-3-ols/procyanidins). The structural analysis of some phenolic compounds was carried out by ion trap mass spectrometer equipped with an electrospray source of ionization in negative mode (LC-ESI-MS). The commercial apple juice contains only four phenolic compounds, with weak concentrations compared to the fresh juice. Lastly, the estimate of the total antioxidant capacity was determined by the FRAP method. The values of the antioxidant capacity in the case of the fresh juices oscillate between 1758 and 3950 µM. In the case of the commercial juice, the obtained results prove that these drinks contain weak capacities of antioxidants compared to the fresh juice.

Keywords: Apple juice, Polyphenols, RP-HPLC, Thiolysis, LC-ESI-MS, FRAP

* Corresponding Author; E. Mail: hedi.hammouda@yahoo.fr
Tel: +216 25124568

1. Introduction

The apple (Malus sieversii) is consumed throughout the world as fruit of mouth or as juice and ciders. This fruit is perceived like a beneficial food for human health. Apple contains simple oses (fructose, glucose, saccharose), sorbitol, organic acids, nucleic acids, minerals, vitamins and...
polyphenols (Demigné, 2002). In apples and apple products (juices and ciders), phenolic compounds contribute significantly to their quality and diversity. Polyphenols are largely implied in astringence, bitterness and color criteria that are essential in the definition of the type and the diversity of apple juices (Lea, 1990).

In apple juice, phenolic compounds show a great diversity of structures and can be classified in four important classes: The flavan-3-ols (F3ols) are subdivided in monomeric forms (catechins) and oligomers and polymeric forms (procyanidins PCA). The catechins are mainly in (-)-epicatechin (EC) and (+)-catechin. Procyanidins structures differ according to the nature and the number of constitutive units, the position and the stereochemistry of the bonds that link the catechin units (Thompson et al. 1972; Thompson, 1988). Generally, this class concentration in cider apple is highest (Sanoner et al. 1999). Hydroxycinnamic acids (second class) included mainly caffeoylquinic acid and p-coumaroylquinic acid (PCQ). The dihydrochalcones class is specific to the apples (Herrmann, 1990). Phloridzin (PLZ), phloretin (PLT) and phloretin xyloglucoside (XPL) are the principal compounds of this class.

Lastly, the flavonols which are quantified by weak concentrations should not be neglected, since it is specific to apples.

Thiolysis coupled to reversed phase High Performance Liquid Chromatography (RP-HPLC) was chosen as method to analyze the procyanidins (Betts et al. 1967) because it is better than a simple assay of total procyanidins. It also gives access to the nature and proportions of the constitutive units and to the average degree of polymerization (DPn).

In addition, some individual molecules of the procyanidin class, for which standards were not available, were characterized by liquid chromatography coupled to mass spectrometry equipped with an electrospray ionization source (LC-ESI-MS) used in the negative mode (Lazarus et al. 1999).

In order to compare the fresh and commercial apple juice, a physicochemical study was undertaken on six varieties of the fresh juice and three varieties of the commercial juices.

This study aims precisely at defining the polyphenolic profile of the six varieties of the fresh apple juice. Two among them are local varieties, compared to with profiles of three commercial apple juice. The total antioxidant capacity of each apple juice is evaluated with the FRAP method and the determination of these correlations with quantities of total polyphenols.

2. Materials and Methods

2.1 Solvents, reagents and phenolic standards

Sodium fluoride (Prolabo, Fontenay-sous-bois, France), acetic acid (Biosolve, Fisher chemicals HPLC grade), Acetonitrile (Prolabo, HPLC gradient grade). Anhydrous methanol (Prolabo, HPLC grade). Toluene-α-thiol and hydrochloric acid were from Merck (Darmstadt, Allemagne). Formic acid was from Fischer scientific, (Analytical grade, Loughborough, UK).

TPTZ, 2,4,6-tripyridyl-s-triazine (Flucka Chemicals Suisse). Acetic acid glacial was purchased from Biosolve Ltd (Valkenswaard, Hollande). Sodium acetate (Merck, Darmstadt, Allemagne). FeCl₃ 6H₂O pur 99-102% (Flucka Chemicals Suisse). FeSO₄ 7H₂O pur 99.5% (Merck, Darmstadt, Allemagne). Ultrapure water was obtained by using a Milli-Q water system (Millipore, Bedford, MA).

Phenolic standards: (-)-epicatechin (EC), (+)-catechin (CAT), 5-O-caffeoylquinic acid, p-coumaroylquinic acid, Quercetin and Phloridzin were obtained from Sigma (Sigma –Aldrich, Germany). Procyanidin B1, B2 (Extrasynthese, Lyon,
Table 1. Physicochemical study of the fresh and commercial apple juices.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>pH</th>
<th>Sugar rate (°Brix)</th>
<th>Index of refraction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fresh juice</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>3.12 (0.03)</td>
<td>7.83 (0.25)</td>
<td>1.3446 (0.0005)</td>
</tr>
<tr>
<td>C</td>
<td>3.09 (0.02)</td>
<td>7.00 (0.50)</td>
<td>1.3433 (0.0005)</td>
</tr>
<tr>
<td>G</td>
<td>4.30 (0.02)</td>
<td>10.83 (0.50)</td>
<td>1.3486 (0.0010)</td>
</tr>
<tr>
<td>A</td>
<td>3.22 (0.03)</td>
<td>8.83 (0.25)</td>
<td>1.3456 (0.0005)</td>
</tr>
<tr>
<td>L</td>
<td>4.09 (0.02)</td>
<td>8.33 (0.25)</td>
<td>1.3456 (0.0005)</td>
</tr>
<tr>
<td>LG</td>
<td>3.80 (0.01)</td>
<td>6.16 (0.25)</td>
<td>1.3423 (0.0005)</td>
</tr>
<tr>
<td><strong>Commercial juice</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>4.28 (0.02)</td>
<td>12.00 (0.00)</td>
<td>1.3516 (0.0005)</td>
</tr>
<tr>
<td>P</td>
<td>4.26 (0.03)</td>
<td>14.50 (0.00)</td>
<td>1.3546 (0.0005)</td>
</tr>
<tr>
<td>E</td>
<td>3.73 (0.01)</td>
<td>15.00 (0.00)</td>
<td>1.3556 (0.0005)</td>
</tr>
</tbody>
</table>

*Z = Zina ; C = Chahla ; G= Golden; A= Anna ; L= Lorca ; LG= Local Golden.; O= Oh! ; P=Plus; E=Enjoy. b Standard deviation (n=3)

Table 2. Concentration of the phenolic compounds (mg/L of fresh juice).

<table>
<thead>
<tr>
<th>Phenolic compounds b</th>
<th>Varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxycinnamic Acids</td>
<td></td>
</tr>
<tr>
<td>CA</td>
<td>359.6</td>
</tr>
<tr>
<td>PCQ</td>
<td>14 3.1</td>
</tr>
<tr>
<td>PCQ_Iso1</td>
<td>3.2 0.06</td>
</tr>
<tr>
<td>PCQ_Iso2</td>
<td>31.4 2.4</td>
</tr>
<tr>
<td>Flavan-3-ols</td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>12.1 0.3</td>
</tr>
<tr>
<td>EC</td>
<td>186.8 15.1</td>
</tr>
<tr>
<td>B1</td>
<td>19.7 1.00</td>
</tr>
<tr>
<td>B2</td>
<td>53.2 1.5</td>
</tr>
<tr>
<td>C1</td>
<td>175.1 6.5</td>
</tr>
<tr>
<td>PCA_X1</td>
<td>nd -</td>
</tr>
<tr>
<td>PCA_X2</td>
<td>12.4 0.9</td>
</tr>
<tr>
<td>PCA_X4</td>
<td>nd -</td>
</tr>
<tr>
<td>PCA_X5</td>
<td>20.3 0.9</td>
</tr>
<tr>
<td>Dihydrochalcones</td>
<td></td>
</tr>
<tr>
<td>PLZ</td>
<td>35.2 0.8</td>
</tr>
<tr>
<td>XPL</td>
<td>95.8 6.00</td>
</tr>
<tr>
<td>Flavonols</td>
<td></td>
</tr>
<tr>
<td>AVI</td>
<td>13.4 0.97</td>
</tr>
<tr>
<td>QCI</td>
<td>7.3 0.34</td>
</tr>
<tr>
<td>IsoQ</td>
<td>5.8 0.75</td>
</tr>
<tr>
<td>HYP</td>
<td>8.8 0.6</td>
</tr>
<tr>
<td>REY</td>
<td>2.4 0.2</td>
</tr>
<tr>
<td>RUT</td>
<td>1.5 0.2</td>
</tr>
</tbody>
</table>

^a Varieties : Z = Zina ; C = Chahla ; G= Golden; A= Anna ; L= Lorca ; LG= Local Golden.
^b CA : caffeoylquinic acid; PCQ : para coumaroylquinic Acid; PCQ_Iso1 and PCQ_Iso2 : isomers of para coumaroylquinic acid; CAT : (+)-catechin ; EC : (-)-epicatechin ; B1 : procyanidin dimers B1; B2 : procyanidin dimers B2; C1 : C1 : procyanidin trimers C1 ; PCA_X1 to X5 : isomers of procyanidins ; PLZ : phlorizin ; XPL : phloretin xyloglucoside; AVI : avicularin; QCI : quercitrin ; IsoQ : isoquercitrin; HYP : hyperosid ; REY : reynoutrin ; RUT : rutin. Standard deviation (n=3); nd : not detected.
France). (-)-Epicatechin benzylthioether used for calibration was purified in the laboratory.

### 2.2 Plant Materials

The products used in this study are apple juice of six Tunisian apple varieties (Zina, Chahla, Golden, Anna, Lorca, Local Golden), harvested during the 2014 season in the areas of Jedeida (North-East of Tunis) and Sbiba (center of Tunisia) and three varieties of commercial apple juice (Oh!, Plus, Enjoy). Before treatment, the fresh apples were washed with pure water. In order to give an account of the variability of the vegetable material, the analysis of polyphenols juice was carried out on three batches for each variety according to the method described by Guyot et al. 1998. For each varieties, three batches of fruits (1 kg) were constituted. Each batch was milled by a Record crusher (type 10, Blaumeyer, France). A solution of diluted sodium fluoride (1 g/L in water) was added to the apple pulp before pressing to obtain juices without oxidation. Then, apple juices were centrifuged (6000 tr/min for 15 min) to obtain clear apple juices. The samples are then freeze-dried.

### 2.3 Freeze-drying of apple juice

For each batch, volumes of 500 µL of the fresh and commercial juices are introduced into tubes of 5 ml. The samples are ready to be freeze-dried by Lyophilisateur LEYBOLD, model LYOVAC WP 2. After 3 days, freeze-drying will be finished.

### 2.4 Physicochemical study

Before proceeding to the identification and quantification of the phenolic compounds, a physicochemical study of the juices of the varieties studied, imposes itself. Indeed, some criteria such as the pH and sugar rates (Brix) have a great relationship with the stability of the bioactive contents found in fruits (Zulueta et al. 2007). The measurement of pH is done directly by using a pH-meter (Ω Metrohm). The index of refraction and the sugar rate are directly given with room temperature (22°C) on the fresh and commercial apple juices by the refractometer (Sopelem, France).

### 2.5 RP-HPLC of methanol extracts and thiolysis reaction medium

The samples were injected onto a RP-HPLC system including an automatic injector model WISP 717 (Waters, Milford, USA) thermostated at 4°C, a gradient pump Model 600 (Waters) and a diode array detector model 996 (Waters). The column was on a RP18 Purospher endcapped 5 µm, 80 Å, 4 x 250 mm (Merck, Darmstadt, Allemagne) equipped with a guard column 4 x 4 mm of the same RP material and thermostated at 30°C. The solvent system was a gradient of solvent A (aqueous acetic acid, 2.5% v/v), and solvent B (acetonitrile of gradient quality). The following gradient was applied at a constant flow rate of 1 ml/min: initial, 3% B, 0-5 min, 9% B linear; 5-15 min, 16% B linear; 15-45 min, 50% B linear followed by washing and reconditioning the column. The solvents were degassed with helium bubbling at a value of 30 % of time per minute. The volume of injection was 10 µL.

The piloting of acquisition, the integration and the processing of the signal were carried out using the Millennium software 2010 version 2.1. Simultaneous monitoring was performed at 280 nm (Flavan-3-ols and dihydrochalcones), 320 nm (hydroxycinnamic acids), and 350 nm (flavonols). Spectra were recorded from 200 to 600 nm (Guyot et al. 1998).

Precisely 500 µL (freeze-dried juice) of the fresh and commercial juices were extracted by 1 mL of pure methanol containing 1 % v/v acetic acid for 15 min in an ultrasonic bath (Brasson 2200, USA). Then, the mixture was filtered on PTFE filters (0.45 µm, Uptidisc interchim, France). The filtrate was ready for HPLC analysis.
2.6 Thiolysis reaction applied on freeze-dried of the apple juices

The thiolysis reaction leads to the depolymerization of procyanidin structures by converting the flavanol extender units into their carbocations and the lower units into monomeric flavanols. The carbocations immediately combine with toluene-α-thiol, leading to the formation of thioether adducts (Rigaud et al. 1991). By making the distinction between terminal and extension units, HPLC analysis of thiolysis media determines the nature and the proportion of the constitutive units of procyanidins. Moreover, their average degree of polymerization (DPn) can eventually be calculated.

Freeze-dried sample (500 µL of the fresh and commercial juices) were added 800 µL to toluene-α-thiol 5% in methanol (v/v) and 400 µL of methanol acidified by HCl (0.4 N). After homogenization, the samples have been carried to 40 °C in a bain-marie for 30 min. The thiolysis reaction has been stopped by a thermal shock in ice crushed for 5 min. The mixture was filtered on PTFE filters (0.45 µm,
Uptidisc interchim, France). The filtrate was ready for HPLC analysis.

The terminal units are directly released in their catechic form, the calculation of the average degree of polymerization (DPn) of the flavan-3-ols is given by the relation (1).

\[
\text{DPn flavan-3-ols} = \frac{\text{Total flavan-3-ols}}{[\text{T_CAT} + \text{T_EC}]}
\]

(1)

\[ [\text{T_CAT}] = \text{concentration in total (+)-catechin obtained after thioacidolyse.} \]

\[ [\text{T_EC}] = \text{concentration in total (-)-epicatechin obtained after thioacidolyse.} \]

\[ [\text{T_EBTE}] = \text{concentration in (-)-epicatechin benzylthioether (extension units) obtained after thioacidolyse.} \]

For a pure procyanidine, the selective quantification of these two types of unit gives access to the determination of the DPn of procyanidin according to relation (2).

\[
\text{DPn procyanidins} = \frac{\text{Total Procyanidins}}{([\text{T_CAT} + \text{T_EC}] - [\text{CAT} + \text{EC}])}
\]

(2)

\[ \text{Total procyanidins} = \text{Total flavan-3-ols} - [\text{CAT} + \text{EC}] \]

\[ [\text{CAT}] = \text{concentration in total (+)-catechin.} \]

\[ [\text{EC}] = \text{concentration in total (-)-epicatechin.} \]

2.7 Identification of some phenolic compounds by LC-ESI-MS in the negative mode

500 µL of the freeze-dried juice were suspended in 1 mL of pure methanol containing 1% acetic acid (v/v) and extracted for 15 min in an ultrasonic bath (Brasson 2200, USA). Then, the mixture was filtered by PTFE filters (0.45 µm, Uptidisc Interchim, France). The filtrate was ready for LC-MS analysis.

The relative acidity of the phenolic protons involves a better detection of the pseudo-molecular ions in negative mode. Phenolic compounds are detected in negative mode in the form of pseudo-molecular ion [M-H]-. The separation of the compounds was carried out on a chain HPLC composed of a system of degasification SCM1000 (ThermoQuest, San Jose, CA, USA), an automatic system of injection (ThermoFinnigan, San Jose, CA, USA), a binary pump Series 1100 (Agilent Technologies, Palo Alto, CA, USA), and photodiode array detector Spectra system UV6000LP (ThermoFinnigan, San Jose, CA, USA). The mass spectrometer was an ion trap LCQ Deca (ThermoFinnigan San Jose, CA, USA) equipped with a source of Electrospray ionization (SIE). The samples injected (5 µL) are separated on a column Zorbax Eclipse XDB-C18 (2,1 mm x 150 mm, 3,5 µm, Agilent Technologies) whose temperature was maintained at 30 °C. The MS spectra were acquired in the “Full Scan” mode in the 50-2000 m/z range. The source parameters were as follows: spray voltage (5 kV), capillary voltage (12.0 V), sheath gas (67 arbitrary units), auxiliary gas (5 arbitrary units), capillary temperature (240 °C). Nitrogen was used as the nebulizing gas. Helium gas was used as a collision gas and the potential change defining the collision energy was optimized in the range of 25-35 V to optimize the production of both parent and daughter ions. Full scan spectra were acquired from m/z 100-1000. The model solutions were first analysed in the full MS mode. The HPLC gradient conditions were as follow: solvent A (aqueous formic acid, 0.1% v/v) and solvent B (acetonitrile containing 0.1% formic acid v/v). with a flow rate of 0.2 mL/min; initial, 3% B; 0-5 min, 9% B, linear; 5-15 min, 16% B, linear; 15-45 min, 50% B, linear followed by washing and reconditioning the column to the initial conditions. The volume of injection was 3 µL.

2.8 Ferric Reducing/antioxidant Power Assay (FRAP)

With acid pH, the reduction of the colorless ferricypridyltriazine complex (FeIII-TPTZ) in its blue
ferrous-tripyrildyltriazine form (Fe\textsuperscript{II}-TPTZ) can be measured following the change of absorbance at 593 nm. This change is directly linked to the oxidation of the antioxidants present in the medium. The given values of FRAP were described by Jiang et al. 2006.

Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl\textsubscript{3} 6H\textsubscript{2}O at 10:1:1 (v/v/v) (Khanizadeh et al. 2008). To 10 \( \mu\)L of white (ultrapure water) or standard (FeSO\textsubscript{4} 7H\textsubscript{2}O (1000 \( \mu\)M)) or samples, are added 90 \( \mu\)L ultrapure water and 200 \( \mu\)L of FRAP reagent (The plate was incubated at 37 °C for the duration of the reaction). The absorbance was taken at 593 nm immediately after and at 1-min intervals up to 4 min using a UV-vis microplate kinetics reader (Multichannel spectrophotometer SPECTRAMax PLUS, Model 384). Change of absorbance \( \Delta A_{593nm} \) of a sample is translated into a value of FRAP by proportionality with that of a solution of a standard (FeSO\textsubscript{4}) of known concentration (Fe\textsuperscript{2+}, 1000 \( \mu\)M). The data were collected and treated using SOFT max Pro 3.0 software.

3. Results and discussion

3.1 Measure pH, rate of sugar and index of refraction of the fresh and commercial apples juices

For the various samples of the fresh and commercial apple juice, the values of pH oscillate between 3.09 and 4.30 (Table 1). This acidity is depending on the presence of several acids such as the hydroxycinnamic acids and especially to the malic acid which is the majority compound in the fresh apple juice. These results are in agreement with those found by Noci et al. 2008. Values of index of refraction vary between 1.3423 and 1.3556 (Table 1). In the case of the fresh apple juice, the rate of sugar varies between 6.16 and 10.83 units Brix. These relatively weak values are compared to those found in the orange juice (Kelebek et al. 2008). In the case of the commercial apple juice the rates of sugar are rather high, probably because they are proportioned by synthesized sugars (Table 1).

3.2 Phenolic compounds of the apple juices

Characterization of phenolic compounds by LC-ESI-MS in the negative mode

Identification of the phenolic compounds was carried out by LC-ESI-MS. Then, in comparison with available standards and interpretation of the MS and UV-visible data corresponding to the main chromatographic peaks were used to identify or characterize the main phenolic compounds in the apple juices samples. Specific pseudomolecular ions corresponding to hydroxycinnamic acids, flavanols, flavonols and dihydrochalcones are selected from the Full MS data. Figure (1) presents an example of an LC-MS extracted ion chromatogram of fresh apple juice methanol extract (Chahla variety). 5-O-Caffeoylquinic acid is detected in class of hydroxycinnamate acid, the [M-H] \textsuperscript{-} ion corresponded is with m/z 353. Five flavan-3-ols were detected, (−)-epicatechin, procyanidin B1, B2, C1 and procyanidin tetramers were also detected by their pseudomolecular ions [M-H] \textsuperscript{-} at 577, 865, and 1153, respectively. Two dihydrochalones, namely phloridzin and phloretin xyloglucoside were also detected in fresh apple juice methanol extract. These compounds showed deprotonated molecules [M-H] \textsuperscript{-} at m/z 435 and 567, respectively. The main flavonols detected in fresh apple juice were isoquercitrin, hyperoside and avicularin. In the case of apple commercial juice, the analysis by LC-ESI-MS shows us the existence of three phenolic compounds 5-O-cafeoylquinic acid, para-coumaroylquinic acid and (−)-epicatechin. Figure (2) is consigned Full MS extracted ions chromatogram of apple commercial juice methanol extract (variety Oh!).

The comparison between the two chromatograms (fresh and commercial apple juices) shows well the
Figure 1. Full MS extracted ions chromatogram of apple juice methanol extract (variety Chahla). Specific pseudomolecular ions corresponding to hydroxycinnamic acids, flavanols, flavonols and dihydrochalcones are selectively extracted from the Full MS data: (1), procyanidin dimers B1; (2), 5-O-caffeoylquinic acid; (3), procyanidin dimers B2; (4), (-)-epicatechin; (5), procyanidin trimers C1; (6), procyanidin tetramers (7), isoquercitrin; (8), hyperoside; (9), avicularin; (10), phloretin xyloglucoside; (11), phloridzin.

Figure 2. Full MS extracted ions chromatogram of apple commercial juice methanol extract (variety Oh'). Specific pseudomolecular ions corresponding to hydroxycinnamic acids, flavanols, flavonols and dihydrochalcones are selectively extracted from the Full MS data: (1), 5-O-caffeoylquinic acid; (2), para coumaroylquinic acid; (3), (-)-epicatechin.
richness of the fresh apple juice in phenolic compounds, and at the same time, the poverty of the commercial apple juice in these compounds.

**Concentrations of the phenolic compounds in the apple juices**

Quantification of the phenolic compounds of the apple juices were carried out by RP-HPLC, the results are expressed out of mg·L⁻¹ of juice. In the cases of the fresh apple juice, the results show that, except in the two varieties Lorca and Local Golden, the flavan-3-ols correspond to the prevalent concentrated class, those varying from 216.5 to 918.7 mg·L⁻¹ of fresh juice for the Local Golden and Chahla variety, respectively (Table 2). (-)-epicatechin was also present in significant amount in the juice of Golden variety (448.7 mg·L⁻¹ of fresh juice). From a quantitative perspective, (+)-catechin has always been considered as a minority compared to (-)-epicatechin. These results are in total agreement with the literature whatever the varieties (Guyot et al. 2003; Lu and Foo, 2000; Mosel and Herrmann, 1974). These results shows the exceptional cases of Lorca and Local Golden variety classified as being strongly concentrated by the class of the hydroxycinnamic acid, respectively 589.7 and 1413.2 mg·L⁻¹ of fresh juice. The juice of the Local Golden variety is characterized by a strong caffeoylquinic acid content (993.3 mg·L⁻¹) accounting for 37.2% of total polyphenols identified by RP-HPLC.

The Golden variety presents the weakest concentration (111.75 mg·L⁻¹ of fresh juice) in dihydrochalcones and the highest was observed for the Golden variety (267.4 mg·L⁻¹ of fresh juice). In all the studied varieties, the flavonols correspond to the minority class, since it is present in concentrations varying from 5.23 mg·L⁻¹ of fresh juice for the Golden variety with 804 mg·L⁻¹ of fresh juice for the Local Golden variety. In apples, flavonols, mainly of esters of quercetin (Teuber et al. 1978), are primarily localized in the peels (Burda et al. 1990; Oleszek et al. 1988). The concentrations of the various phenolic classes in the considered apple juices are represented by the histogram (Figure 3).

The results also highlight the great diversity of the phenolic composition with total polyphenols contents (identified by HPLC) according to the varieties. The Golden variety for instance has the lowest content (378.6 mg·L⁻¹ of fresh juice); whereas the Local Golden has the highest content (2667 mg·L⁻¹ fresh juice). The histogram (Figure 4) represents the total polyphenols contents in the fresh apple juices.

The commercial apple juice contains only four phenolic compounds endowed with a concentrations compared to the fresh juice. The results show that caffeoylquinic acid is present in the commercial juices by weak concentration, and to compare between the commercial juices it is interesting to note that the Oh! variety has the highest concentration in caffeoylquinic acid (34.8 mg·L⁻¹ commercial juice) compared to the Plus and Enjoy varieties (Table 3). One can also say that (-)-epicatechin is more concentrated in the Oh! variety, but by low values (26 mg·L⁻¹ commercial juice) compared to the fresh juices.

The apple juices are potentially rich products in polyphenols and, particulary, in procyanidins (Guyot et al. 2003). Under manufacture, the procyanidins are partly implied in reactions of oxidation, along with the formation of new structures having different properties and, probably of new implications for their contribution to the organoleptic and nutritional qualities.

### 3.3 DPn of flavan-3-ols and Procyanidins of the fresh and commercial apples juices

In the fresh apple juices, the average degrees of polymerization of the flavan-3-ols (DPn F3ol), generally, vary from 2.0 to 3.7 (Table 4). This means that the flavan-3-ols are mainly present in the oligomers forms (2 <DPn< 6) (Lea and Arnold, 1978;
Sanoner et al. 1999). For some apple juice varieties (Golden), the average degree of polymerization of the procyanidins (DPn PCA) presents exceptionally a high value (24.9). As, for the other varieties, the procyanidins are present in oligomers forms. Lea and Arnold (1978) studied the relation between DPn of the procyanidins and the properties of these molecules, in particular, the implication of the procyanidins in the feelings of bitterness and astringency. These authors mention that the procyanidins oligomers (2 <DPn< 6) are mainly responsible for the bitterness while the high polymeric procyanidins are implied in the feeling of astringency.

It is remarkable that in the commercial juices, it (-)-epicatechin is only the representative identified in the class of the flavan-3-ols, therefore there exists neither of DPn F3ols, nor DPn PCA.

3.4 Antioxidant capacity of the apple juices by the FRAP method.

The role of polyphenols, and in particular of the procyanidins, is mainly related to their capacity to

**Figure 3.** Concentrations of the phenolic classes in the fresh juices (mg/L of fresh juices).
Varieties: V1 = Zina; V2 = Chahla; V3 = Golden; V4 = Anna; V5 = Lorca; V6 = Local Golden.

**Figure 4.** Concentrations of the total polyphenols in the fresh juices (mg/L of fresh juices).
Varieties: V1 = Zina; V2 = Chahla; V3 = Golden; V4 = Anna; V5 = Lorca; V6 = Local Golden.
interact with others compounds (proteins, polysaccharides...), and with their antioxidant capacity. Nevertheless, total antioxidant capacity can be partly attributed to non-polyphenolic compounds with antioxidant character (Haslam, 1996; Haslam et al. 1988; Treutter, 2006).

The values of the antioxidant capacity in the case of the fresh juices oscillate between 1758 and 3950 µM (Table 5) in the Anna in Lorca variety, respectively. In the case of the commercial juice, the obtained results prove that these drinks contain weak antioxidant capacities compared to the fresh juice. One can also say that the total antioxidant capacity can be due not only to polyphenols, but also to other chemical compounds in antioxidant matters such as the vitamin C additive used as agent of conservation can contribute to this capacity (Singleton and Rossi, 1965).

4. Conclusion

In this study, we showed the great difference between the fresh and commercial juices at the level of phenolics profiles and especially DPn of the flavanols and procyanidines. The phenolics profile of the fresh juices comprises more than twenty compounds distributed out of four phenolics class. Four compounds in the case of the commercial juices were detected. These differences can be associated with the procedure of preparation of these juices (no protection of oxidation) and probably related to the procedures of filtration or clarification. The results of DPn of the flavanols and procyanidines are normally obvious because the DPn are related to the concentration of the flavanols and the procyanidines in the extracts, because in the commercial juices one does not find procyanidins. The antioxidant capacity is related to the content of bioactive molecules, although we found antioxidant capacities in the commercial juices. This result is probably related to the existence of the added substances in these juices like some vitamins or polysaccharides, since if the results of the physico-chemical study are examined at it is found that the sugar content is higher in the commercial apple juices.

References


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