# FROM ROMANIAN APPLE TO JUICE AND APPLE CIDER - A COMPARATIVE STUDY AND PHYSICOCHEMICAL ANALYSES 

Dana BOGDĂNESCU ${ }^{1}$, Despina Maria BORDEAN ${ }^{1}$, Mariana-Atena POIANĂ ${ }^{1}$, Nicoleta HĂDĂRUG ${ }^{1}$, Ioan TĂRANU ${ }^{2}$, Adrian RIVIȘ ${ }^{1}$

e-mail: dana_bogda@yahoo.com


#### Abstract

Romania has a long tradition in the production of high quality apples, which can be processed into a large variety of soft drinks. The aim of the study was to establish the physicochemical characteristics of some apple varieties growing in Romania, together with their corresponding juice and cider, regarding polyphenols, antioxidant capacity, pH and refractive index. Some samples of apple, apple juices and corresponding ciders were analysed using Folin Ciocalteu method for total polyphenols content (TPC) and CUPRAC method to evaluate the antioxidant capacity (TAC). For all cases, moisture content, sugars (like ${ }^{\circ}$ Brix), pH and alcoholic content were recorded. Compared to fresh apples, a significant decrease of the antioxidant capacity measured by CUPRAC assay and TPC for cider was observed. Also, statistically significant differences between samples were found. During processing of apples to juice and cider, a difference in the antioxidant capacity of the final product was noticed. Our study shows that processing apples to juice and further to cider has the effect of drastically decreasing the TAC to $2-6 \%$ of the initial apples TAC, depending on the apple variety.


Key words: antioxidant capacity, total polyphenols content, Romanian apple cider.


#### Abstract

Romania has a long tradition in the production of quality apples, which can be processed into various soft drinks.

Fruits and vegetables contain several healthpromoting factors, including fiber and high concentrations of phenolic acids, flavonoids, vitamins, and minerals. Phenolic acids and flavonoids, may provide long term protection against a number of chronic diseases (Verdu, 2013).

Polyphenols are abundant micronutrients in diet, and evidence for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging. The health effects of polyphenols depend on the amount consumed and on their bioavailability (Manach, 2004).

This information can be used for product and process optimization (Van Der Sluis, 2002). The antioxidant capacity of individual phenolic compounds, however, may vary significantly depending on the chemical characteristics and assay mechanisms used (Tsao, 2005). The acceptance of fruit to consumers is determined by


visual attributes that include aspect, size, uniformity, color and freshness, as well as nonvisual attributes such as taste, aroma, flavor and firmness (Geddeda, 2014)

The total flavonoid content in some apple varieties is in range from 26.4 to $73.9 \mathrm{mg} / \mathrm{g}$ fresh weight and is not related to fruit size (Price, 1999). The average content of total polyphenols in the apples evaluated by the Folin Ciocalteu assay was $110.2 \mathrm{mg} / 100 \mathrm{~g}$ of fresh fruit with significant differences depending on the apple variety. Rennet apples have a much higher content of total polyphenols than any other variety (Vrhovsek, 2004). The effect on antioxidant capacity of processing apples into juice was studied. Raw juice obtained, for example from Jonagold apples, had an antioxidant capacity that was only 10 and $3 \%$ of the fresh apples.

Total antioxidant capacity information can be used in conjunction with existing databases in order to better describe acceptable attribute ranges of authentic apple juice for the development of commercial apple varieties that would target specific consumer requirements (Eisele, 2005).

[^0]Alcoholic fermentation of the juice is the basic cider producing technique. Apples are first chopped and squeezed to generate juice and then that juice is fermented to produce cider.

Polyphenols from apples are largely responsible for cider quality (Sanoner, 1999).

Cider and apple wines are traditional products in some countries (Merwin, 2008). In European countries such as England, France and Germany, but also in United States and Canada cider and apple wine are produced in significant quantities, although a considerable proportion is also distilled to make apple brandy. In France, apple brandy is known under the brand name Calvados - protected since 1942 (Merwin, 2008). In Great Britain and France, the term "cider" (or in French "cidre") means apple wine, hard cider, or fermented apple juice. On the other hand, in the United States it may mean fermented or unfermented apple juice based on the definitions and available products. Broadly, it can be classified as soft cider with $1.5 \%$ alcohol content, hard cider with 5-8\% or apple wine with $8 \%$ up to 14\% (Kosseva, 2016).

Basic tasting perceptions such as bitterness, sweetness, sourness, and astringency (Joshi, 2006) are elicited by groups of compounds including glycosides, dipeptides, sugars, acids, alkaloids, and phenols characterized by low volatility (Kosseva, 2016). The polyphenols are predominantly responsible for bitterness and color since they are also linked to the quality of apple juices and also of cider (Verdu, 2013). Through the formation of complexes with salivary proteins, condensed tannins are responsible for the astringent character of fruit (grapes, peaches, kakis, apples, pears, berries, etc.) and beverages (wine, cider, tea, beer, etc.) (El Gharras, 2009).

The cider has been extolled as a therapeutic agent. It is an important addition to human diet, containing phenols and other bioactive compounds with antioxidant activities (Wojdylo, 2008). Some researchers consider that the results for the antioxidant capacity of solutions should be compared only if they have the same pH or if it has been proven that they consist of just pH independent antioxidants (Lichtenthaler, 2005).

Cider parameters monitored throughout the fermentation included: organic acid content, titrable acidity, pH , ethanol production, and sugar content (Reuss, 2010). Phenol content controls both microbiological spoilage and guarantees flavor quality of ciders (Mangas, 1999). The juice/cider maker can predict the quality of the obtained product by inspired choice, based on the physicochemical characteristics of the apple varieties used in the process (Alberti, 2016).

The aim of the study was to consider the physicochemical characteristics of some apple varieties growing in Romania in terms of cider manufacturing. The evolution of some features such as: polyphenols, antioxidant capacity, refractive index and pH on the chain apple - juice - cider were followed. Some useful milestones for those who want to produce cider for personal or industrial use have been drawn.

## MATERIALS AND METHODS

Ten apple varieties growing in Romania were used as raw materials for fermentation medium: Granny Smith (GS), Starkrimson (SK), Golden Delicious (Gd), Florina (Fl), Rennet (Re), Jonathan (lo), Idared (Id), Reghin Pinova (Rp), Kalter (Ca), Yonagold (Yg). According to the literature, these varieties are frequently used worldwide in cider production and fortunately they are varieties that are now cultivated in Romania as well. Some of them have been cultivated for a long time in our country - like Jonathan or Reghin Pinova - and the others have been introduced recently in Romanian orchards. Because the characteristics of the fruits depend on specific local factors such as soil composition, climate, treatments applied, etc. it is expected that the fruits obtained in Romania will differ from those grown in other parts of the world. That is why it is necessary for the fruits grown in Romania to be studied in terms of their use in the production of cider.

The harvested fruits were transported to the processing unit and stored at $10^{\circ} \mathrm{C}$ - just to the maximum maturation degree - to ensure the highest level of fermentable sugars. 15 days storing was done in order to ensure the fruit's similarity in quality and even a slight dehydration effect. This provides the increase in the sugar content, which creates the premises of obtaining a superior quality cider with high alcohol content. In the cider process the most important is the quality of apples and not the productivity, so that the light weight loss at this stage of maturation is not critical.The apple samples were delivered by different producers from Banat and Transylvania in Romania (crop 2016).

First, the apples were selected by appearance, with no visible damage. Next, they were washed, disinfected, washed again and then rinsed. Clean apples were chopped, squeezed and the resulted juice was filtered by the rough impurities. The brut apple juice was thus obtained. For best cider quality, the juice was clarified with the enzyme RohaPect (PTE 100) and $\mathrm{CaCl}_{2}$ solution, according to the method proposed by Markowski (Markowski, 2005). We used RohaPect 100 pectinylase (PE) - used in the production of fruit distillates. Pectinlyase is derived from a mold of the genus Aspergillus. • IUB-No .: 4.2.2.10 CAS-No 9033-35-6 (AB Enzymes GmbH Darmstadt Germany). The juice treated this way is
left to rest for 24 hours at a temperature of $16^{\circ} \mathrm{C}$, after which $40 \mathrm{ml} / 100$ I juice of a $30 \% \mathrm{CaCl}_{2}$ solution is added. After another 24 hours, the pectin is deposited on the bottom of the rinse pot in a dense layer and the clear layer is separated. For the $\mathrm{CaCl}_{2}$ solution, $\mathrm{CaCl}_{2}$ analytical grade was used from Merck and freshly prepared deionized water.

The clear juice, free of pectin - negative results with pectin test - was ready for final chemical analysis. The pH and refractive index (IR) were measured and finally the yeast was added and fermentation was started. The fermentation was conducted in the presence of Saccharomyces cerevisiae - dry yeast brand Mangrove Jack's specially created for cider manufacturing. During fermentation - at constant $18^{\circ} \mathrm{C}$ - the evolution of ${ }^{\circ}$ Brix was assessed. When it reached $6{ }^{\circ}$ Brix, the cider was placed at lower temperature $\left(4-5^{\circ} \mathrm{C}\right)$. The fermentation process was ended and the yeast and residual insoluble impurities were separated by several decantations. Finally, when the raw cider was clear, it was bottled, labeled and matured in dark place at $10-12^{\circ} \mathrm{C}$. After two months, it was analyzed and tasted.

Chemical reagents - ethanol, Folin Ciocalteu reagent, sodium carbonate, gallic acid, 2,2-diphenyl- 1-picrylhydrazyl (DPPH), 2, 4, 6-tripyridyl-striazine (TPTZ), acetic acid, iron (III) Chloride hexahydrate, 2,2'-azino-bis(3-ethylbenzthiazoline- 6-sulphonic acid) (ABTS), potassium persulfate and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were all of analytical grade and were purchased from Merck. Deionized water was prepared freshly by an inverse osmosis system (max. 7 micro Siemens - level of conductivity).

For alcoholic content ebulliometer DujardinSalleron D.E. 2000 was used.

Refractive index was measured using an ATC Refractometre Brouwland Belgium and for moisture content, a T-balance Sartorius MA 50 Bradford City United Kingdom was used.

For pH measurements was used a Radelkis Budapest - Precision Digital pH Meter type OP 208 and a calibration solution pH 4.01 (Hanna Instruments Szeged Hungary).

All samples of apple, apple juices and corresponding ciders were analysed using Folin Ciocalteu method for total polyphenols content (TPC), at 750 nm , and CUPRAC method to spectrophotometrically evaluate the antioxidant capacity, at 450 nm , using Neocuproine $\geq 98 \%$ (Aldrich), sodium carbonate BioXtra and copper chloride (https://www.oxfordbiomed.com /sites/default/files/spec_sheet/FS02.pdf)

Samples of extracts for the polyphenols analyses of apples were obtained by adding 5 g of apple into 100 ml ethanol p.a. Extraction time was 30 min., under stirring at $20^{\circ} \mathrm{C}$. The procedure is adapted with some minor modification from a procedure developed by Candrawinata, V. I. et al. in 2014.Total phenolic content of the samples were determined according to Folin-Ciocalteu method using gallic acid as a standard (Singleton and Rossi, 1965; Singleton et al., 1999). After addition of Folin-Ciocalteu reagent to the sample solution, it was allowed to react for 6 min . Reaction was stopped using 1.50 mL of $20 \%$ sodium carbonate. The extracts were oxidized with Folin-Ciocalteu reagent, and the reaction mass was neutralized with sodium carbonate. The absorbance of the resulting blue color was developed in 120 min , in a dark place, and the absorbance was measured (Budak, 2015). Total phenol content was expressed as mg Gallic acid equivalent/g - for apple samples and $g$ GAE / I for juice or cider samples - using the equation obtained from the calibration curve for Gallic acid. Data are expressed as mean $\pm$ SD of three replicates (Framarzi, 2014).

## RESULTS AND DISCUSSIONS

For all ten apple varieties studied we recorded the moisture values - presented in table 1.

The lowest moisture was observed for Ionagold $75.81 \%$, the highest for Idared 86.28 \% and an average at $80.17 \%$ was determined.

Table 1.
The apple varieties moisture

| GS U(\%) | SK U(\%) | Gd U(\%) | FI U(\%) | $\operatorname{Re~U(\% )~}$ | lo U(\%) | Id U(\%) | Rp U(\%) | Ca U(\%) | $\mathrm{Yg} \mathrm{U( } \mathrm{\%)}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 77.59 | 78.08 | 75.83 | 82.32 | 80.63 | 81.80 | 86.28 | 78.50 | 84.85 | 75.81 |

In Figure 1 the apple moisture is shown. Statistical processing was done using PAST version 2.14 and MVSP version 3.13 software.

Principal component analysis (PCA) was the multivariable tool applied to sort the samples according to their response values.

Prior to the application of PCA, all the variables were autoscaled to standardise the statistical importance of each variable. By reporting to the GSU and SKU, the moisture of the other analyzed samples can be grouped in three clusters - figure 3.


Figure 1. The apple moisture


Figure 2. Group presentation on the basis of the moisture content.


Figure 3. Three clusters representation using Euclidean - Data square root transformed

Table 2, 3 and 4 show total polyphenol content (TPC) and total antioxidant capacity (TAC) for 7
types of apple juices, fresh juice and respectively cider.

Table 2
Antioxidant capacity (mmol Trolox / g) and content of polyphenols (mg GAE/g fresh apple) depending on the variety of apple

|  | GS | SK | GD | FL | RE | JO | ID |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Apple-TPC | 1.377 | 2.884 | 1.485 | 1.571 | 2.986 | 2.332 | 2.742 |
| Apple-TAC | 259.8 | 277.2 | 227.8 | 156 | 391.8 | 280.8 | 289.4 |

LEGEND: Granny Smith = GS; Starkrimson = SK; Golden Delicious = GD; Florina = FL; Rennet = RE; Jonathan = JO; Idared = ID; Total Polyphenol Content = TPC, Total Antioxidant Capacity = TAC.

Table 3
Antioxidant capacity (mmol Trolox / I) and content of polyphenols (g GAE/l of juice sample) depending on the variety of apple juice ( AJ )

|  | GS | SK | GD | FL | RE | JO | ID |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A J-TPC | 8.895 | 10.794 | 8.916 | 8.994 | 9.504 | 9.258 | 9.48 |
| A J-TAC | 180.3 | 189.10 | 188.60 | 201.10 | 177.40 | 171.50 | 188.00 |

LEGEND: Granny Smith = GS; Starkrimson = SK; Golden Delicious = GD; Florina = FL; Rennet = RE; Jonathan = JO; Idared = ID; Total Polyphenol Content = TPC, Total Antioxidant Capacity = TAC.

Table 4
Antioxidant capacity (mmol Trolox/l) and content of polyphenols (g GAE/I of cider sample) depending on the variety of cider (CY)

|  | GS | SK | GD | FL | RE | JO | ID |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| CY-TPC | 2.322 | 2.29 | 3.33 | 2.762 | 3.171 | 3.03 | 2.92 |
| CY-TAC | 7.76 | 9.13 | 7.24 | 9.01 | 8.34 | 9.85 | 5.95 |

LEGEND: Granny Smith = GS; Starkrimson = SK; Golden Delicious = GD; Florina = FL; Rennet = RE; Jonathan = JO; Idared = ID; Total Polyphenol Content = TPC, Total Antioxidant Capacity = TAC.

From the data presented in Table 2, it is noticed that the largest TPC has Rennet apples and the smallest Granny Smith - 33.49 \% from RE value. All small values of TPC appear at Golden and Florina, with 49.7 and 52.6 \% less than the
maximum values of RE, while very red varieties such as Starkrimson or Ionatan are close to the maximum value of TPC. Although it does not have the intense red bark, Idared still has a high TPC value $-95.9 \%$ toward RE. Florina has a relatively
small TPC (71.5\% toward medium TPC value), although it is much reddish than Golden or Granny Smith. The average TPC for apples analyzed is 2.197.

Analyzing the antioxidant capacity of apples, Rennet has the highest value followed by Idared, Ionatan, Starkrimson and the lowest value is for Florina - only $40 \%$ from the value of Rennet. In this case, the average TAC value is 268.97.

The juices obtained from these apple varieties behave quite differently - the highest value of TPC was observed for Starkrimson 10,794 - and the smallest $(8,895)$ for Granny Smith - which is $82.4 \%$ from SK value. The differences between the TPC values determined for the juice are quite small compared to the apple variety, unlike those observed at apple samples. In terms of TAC, apple juices have close values again ranging from 171.50 for Rennet and 189.10 for Starkrimson. The difference between the lowest and the highest value is only approx. $10 \%$. The mean values for TPC and TAC are 9.406 and 275.44, respectively.

Further processing of the juice and cider production resulted in a TPC of 3.33 for Golden, then to Ionatan (3.03) Idared (2.92) and the lowest value of 2.29 at Starkrimson, compared to an average of 2.415 . The fermentation process leads to a significant decrease in TPC values for cider compared to those found on the appropriate apple juice. The largest decrease is observed to SK cider has $21.22 \%$ of that of SK juice. Of the studied apple varieties, polyphenols in GD best withstand to the fermentation process - $37.35 \%$ of polyphenols in juice are preserved in the GD cider.

SK loses most of TPC fermentation - $78.78 \%$ of the value determined for juice. GS conserving $25.98 \%$ of the TPC determined for apple juice also behaves very well.

The TAC values show that the largest TAC has the cider obtained from Ionatan (9851.98) followed by Starkrimson and Florina and the lowest value at Idared (5957.42). For the analyzed TAC's the mean TAC value is 8185.89 . In terms of TAC, the decrease from the values determined at the apple is much more pronounced. The highest decrease occurs at ID - the cider obtained from the ID has only $2.06 \%$ of the TAC of the apple and the lowest decrease in FL - which holds $4.47 \%$ of the TAC of FL apple. Analyzing the juice - cider chain, it is observed that a significant decrease in TAC occurs at the transition from juice to cider during the fermentation process.

In terms of TPC, the largest amount of antioxidants can be extracted from Rennet apples, Starkrimson apple juice and Golden Cider.

Apples used in this study for cider have the characteristics shown in Table 5.

In terms of pH it is observed that the lowest pH is Rennet (3.28) and the largest 4.2 - Ionathan apple varieties. It is known from the literature and also from practice that the pH of the juice used for cider production must be no more than 3.6. The highest ${ }^{\circ}$ Brix value was measured at Golden Delicious (15.60) and the smallest 10.3 at Idared. Higher sugar content allows for a higher alcohol concentration. That is why it is absolutely necessary to choose judiciously the variety of apples used to obtain the cider.
${ }^{\circ}$ Brix and apple pH values

|  | Apple varieties | pH | ${ }^{\circ}$ Brix |
| :---: | :---: | :---: | :---: |
| 1. | Rennet (RE) | 3.28 | 14.2 |
| 2. | Starkrimson (SK) | 3.95 | 13 |
| 3. | Idared (ID) | 3.4 | 10.3 |
| 4. | Gramy Smith (GS) | 3.79 | 11.5 |
| 5. | Golden Delicious (GD) | 3.79 | 15.6 |
| 6. | Ionathan (IO) | 4.2 | 10.4 |
| 7. | Florina (FO) | 4.02 | 11.7 |
| 8. | SK + ID (1/2) | 3.54 | 11 |
| 9. | SK+GD (1/1) | 3.93 | 13.6 |
| 10. | GD + RE (1/1) | 3.67 | 15 |

## CONCLUSIONS

The analysis of the apples studied show that the lowest moisture is the Ionagold apple
$75.81 \%$, the highest for Idared $86.28 \%$ and having an average of $80.17 \%$.

A significant modification of the antioxidant capacity measured by CUPRAC assay and TPC analysis for cider compared to fresh
apples and apple juice was observed. For apple and apple fresh juice the greatest antioxidant capacity was found.

To obtain a quality cider - in terms of sugar, alcohol and antioxidants content, it is necessary to choose those apple varieties that are also sweet enough - so can provide enough alcohol content and enough residual sugar, but also have a pH value smaller than 3.6. Not to be neglected the polyphenol content of apple or juice that can provide a moderate to maximum antioxidant content in cider.

Although the "polyphenol" criterion is important for cider quality, data on the phenol composition of apple varieties growing in Romania are still poor. They correspond essentially to the global estimation of the polyphenol concentration in apple juice. In some cases, the assay does not give complete information on the "polyphenol potential" of the fruits because an important part of the native compounds are oxidized and adsorbed on the apple cell wall when fruits are processed into juices. The decrease is more significant to TAC than to TPC.

The apples grown in Romania are valuable products, of high quality, so even the highest quality expectations of future cider manufacturers will be met.

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[^0]:    ${ }^{1}$ Faculty of Food Processing Technology, University of Agricultural Sciences and Veterinary Medicine of Banat " KING Mihai I of Romania"Timișoara, 119 Calea Aradului,300645 Timisoara, Romania.
    ${ }^{2}$ National Institute of Research and Development for Electrochemistry and Condensed Matter-Timișoara 144 AP Podeanu, 300569 Timişoara, Romania

