Evaluation of Physical-Chemical Indexes, Sugars, Pigments and Phenolic Compounds of Fruits from Three Apple Varieties at the End of Storage Period

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Abstract

Apples are the most cultivated and consumed fruits in the world. They not only taste great, but there are also rich sources of monosaccharides, pigments, fibers, functional compounds such as polyphenols which are well-known for their antioxidant action. Due to the high level of apples consumption, it is important to monitor and know the detailed chemical composition of this fruits on the market shelf. The aim of this paper was to study the detailed chemical composition of apples from three varieties. Samples from three varieties (Ionathan, Golden Delicious and Starkrimson) were taken from the Romanian market. Individual sugars composition was performed by HPLC, total polyphenols content by Folin Ciocalteu method, antioxidant capacity by using the DPPH test, while pigments were analysed by spectrophotometric specific methods and the total starch content measured by a polarimetric method. Water content, acidity, total soluble solids and pH were also monitored through specific methods. There were found differences between varieties particularly in relation to the polyphenols content, carotenoids and chlorophyll. Regarding the individual sugars composition, fructose and glucose were predominant followed by sucrose for all samples. Values of starch, moisture, acidity, total soluble solids and the pH were according to other apple varieties found in literature. These results provide important information regarding the chemical composition of apple varieties from Romanian market, for both human direct consumption and industrial processing.

Keywords: *Ionathan, Golden Delicious, Starkrimson, apple chemical composition, sugars, pigments, antioxidants.*

INTRODUCTION

The importance of apples in the human diet is well known, global product quantities confirming that they are some of the most popular fruits. In 2011 apples ranked second worldwide, with a production of 2011 ~ 75 million tons (FAO, 2013). In 2011 (the last year reported by the FAO, 2013), in the European Union, in terms of apple production , Romania ranked sixth, with a production of 620.000 tones, close to the Germany and Spain.

Apples are an important part of the human diet because they are a source of monosaccharides, minerals, dietary fiber and various biologically active compounds such as vitamin C, and some phenolic compounds, which are known for their action as natural antioxidants (Miller and Rice-Evans 1997).

Apples are eaten fresh or as compotes, jams, juices, juice concentrates, vinegars, flavorings, or extracts of various chemical compounds existing in the fruit. Apple fruit quality is very important, being determined by the content of sugars, organic acids and phenolic compounds. They have a very important role in taste characteristics such

as flavor, bitterness, astringency and also color (Jihong *et al.*, 2007).

Phenolic compounds concentration is strongly dependent on the apple variety and their maturity, closely related to the sensory and nutritional qualities of fruit. The starch content is an important factor for identifying appropriate maturation of the fruit.

Fruit color is a feature for assessing their quality and at the same time an indicator for assessing the maturity level at harvest (Muste, 2008). The color is a characteristic for species and is determined by the presence of pigments in cells of the epidermis and hypodermis. Interest in the beneficial effects of pigments on human health has stimulated an increase in demand for their use in food and supplementary food products.

Due to the high level of apples consumption as well as their health benefits, it is important to monitor and know the detailed chemical composition of this fruits on the market shelf. The aim of this paper was to study the detailed chemical composition of apples from three varieties from the Romanian market.

MATERIALS AND METHODS

Samples characteristics

Ionathan, Golden Delicious and Starkrimson apple varieties from Reghin region, harvested at technological maturity and stored in cells with a controlled atmosphere (temperature 0-2°C, relative humidity 90-95%, oxygen 1-3%, carbon dioxide 1-2%) for 115 days, were analyzed at consumption maturity.

Moisture Determination

The protocol used was based on AOAC Official Method. Moisture content was determined by drying in an oven at 103°C ± 2 °C for 3 hours, the experiment being repeated until the weight was constant. The samples were cooled in a desiccator for one hour and weighed (AOAC, 1999).

Soluble Solids Determination – Refractometry

The protocol used was based on the standardized ISO 2173:2003. Analyzed apple pulp was grounded with a Philips HR1614/00 650 W vertical blender and passed through a gauze. Samples were analyzed using a Carl Zeiss refractometer. The measured refractive index is related to the amount of soluble solids (expressed as the concentration of sucrose) using the conversion table or by direct reading on the scale of the refractometer (ISO 2173: 2003).

Titratable Acidity Determination

Determinations were made according to the international standard (ISO 750:1998), with some modifications.

pH Determination

This determination is based on the method proposed by Rosnah *et al.* (2012) with minor modifications. The pH was measured using a Hanna Instruments pH meter, prior calibrated to the pH 4.0 respectively pH 7.0, using a buffer solution.

Antioxidant Capacity Determination by DPPH Method

The antioxidant capacity was determined by assessing the effect of elimination of free radicals (Free Radical Scavenging effect) over 1,1-diphenyl-2 -picrylhydrazyl (DPPH) radical. This determination is based on the method proposed by Odriozola-Serrano *et al.* (2008). An amount of 10µl of the methanolic extract from the analyzed samples, obtained according to the method described by Bunea *et al.* (2011), was mixed with 3.9 ml of DPPH (0.025g /l) and 90 µl of distilled water. The mixture was stirred and maintained properly in the dark for 30 min. The absorbance of the samples was measured at 515 nm (Shimadzu 1700 UV-VIS) against a methanol blank. Results were expressed as percent over standard DPPH absorbance.

$$RSA\ [\%] = \frac{A_{DPPH} - A_P}{A_{DPPH}} \times 100$$

RSA [%] - *Radical Scavenging Activity*;
A_{DPPH} – DPPH absorbance;
A_P –sample absorbance.

Total Polyphenols Determination by Folin-Ciocalteu Method

Total polyphenol content in whole apple was determined according to the method described by Cerbu *et al.* (2012). Absorbance was read at 750 nm with a Shimadzu UV-VIS 1700 spectrophotometer. The standard curve was carried out using concentrations of 0, 0.25, 0.50, 0.75, 1 mg/ml of gallic acid. Total polyphenol content in whole apple was expressed in gallic acid equivalents, mg of GAE/100 g FW (Sconta, 2012).

Carotenoid Pigments Determination

Extraction was performed using the procedure described by Bunea *et al.* (2008) with some modifications. Carotenoids were extracted from the freeze-dried apple skins with liquid N_2 (*freeze drying*) using as solvents: methanol, ethyl acetate, petroleum ether (1:1:1, v/v/v). Successive extractions were performed. The extracts were combined, filtered and washed with distilled water, diethyl ether and a saturated solution of NaOH. The ethereal phase was recovered and subjected to rotary evaporation at 35°C. The remaining extract was dissolved in a known volume of methanol and stored at -18 °C until it was subjected to analysis.

Estimation of carotenoids was determined using Shimadzu UV-VIS 1700 spectrophotometer by reading at 450 nm wavelength. Estimation of content of carotenoids was achieved by formula:

$$X\ (mg\ of\ carotenoids) = \frac{A \times V \times 10^3}{2500 \times l \times 100}$$

A = absorbtion at λmax = 450 nm; V = sample volume (ml); 2500 = the molar absorption coefficient (E1%); l = 1 cm – optical path length (Britton *et al.*, 1995).

Chlorophyll Pigments Determination

Apple peel chlorophyll extraction was performed according to the method described by Lancaster *et al.* (1994) with some modifications. Absorbance was measured at 645 and 663nm. Chlorophyll content was calculated using Arnon (1949), equations:

$$Chl\ a\ (mg\ g^{-1}) = [(12.7 \times A663) - (2.6 \times A645)] \times \text{ml acetone / mg sample}$$

$$Chl\ b\ (mg\ g^{-1}) = [(22.9 \times A645) - (4.68 \times A663)] \times \text{ml acetone / mg sample}$$

$$Chl\ tot = Chl\ a + Chl\ b.$$

where: Chl a – clorophyll a; Chl b – clorophyll b; A663 – absorbance at 663 nm; A645 – absorbance at 645 nm; Chl tot – total clorophyll.

Starch Determination by Ewers Polarimetric Method

The starch content of the apple pulp was determined using the Ewers polarimetric method (ISO 10520: 1997) with some modifications. (Cerbu *et al.*, 2011).

Starch content A (% w/w) reported on dry weight was calculated using the formula:

$$A = \frac{2000}{\alpha_D^{20}} \times \frac{2.5\alpha_1}{m_1} \times \frac{100}{w_1}$$

where: : α_1 – the numerical value of optical rotation in read polarimeter [polarimeter degrees]; $\alpha_D^{20}\alpha_D^{20}$ – specific numerical value for the measurement of optical rotation of pure starch [polarimeter degrees]; m_1 –sample weight [g]; w_1 – dry matter content of the determined sample [% w/w].

Individual Sugars Determination by High Performance Liquid Chromatography (HPLC)

Determination of individual sugars by high performance liquid chromatography to determine the concentration of sugar in apple juice samples obtained in the study were made in accordance with Honey Harmonised Methods of the International Commission (2002). The equipment used was a Shimadzu High Performance Liquid Chromatography (HPLC), consisting of controller, auto sampler, degasser, pump, IR detector, and the sugars separation was achieved on a modified Alltima Amnio 100A, 5µm, 250 × 4.6mm column. Mobile phase flow rate was 1.3 ml/min. Column temperature was 30 °C, injected sample volume being 20µl. It was worked on isocratic conditions, using as mobile phase acetonitrile/water (75/25, v/v) mixture.

RESULTS AND DISCUSSIONS

The results on the chemical composition of the three apple varieties are presented in *Table 1* and *Table 2.* Moisture values were between 82.09 and 85.4 %. These values are consistent with values reported by Campeanu *et al.* (2009).

Acidity is different depending on the variety, Ionathan variety register an acidity of 0.29 % followed by Strakrimson variety with an acidity of 0.19 % and Golden Delicious with an acidity of 0.17 %. These values were below the maximum limit of 0.31%. Lower acidity leads to a better acceptance for apple to be consummated. In agreement with our results, Drogoudi *et al.* (2008) reported a higher content of total acidity in pulp

Table 1. Physical-chemical indexes composition of the three apple varieties

	Chemical composition			
Variety	**Moisture (%)**	**Acidity (%)**	**Total soluble (°Brix)**	**pH**
I	85.34 ± 0.17	0.29 ± 0.02	23.25 ± 0.35	3.60 ± 0.02
GD	85.43 ± 0.16	0.19 ± 0.01	19.25 ± 0.35	3.75 ± 0.00
S	82.09 ± 0.28	0.17 ± 0.02	19.75 ± 0.35	4.12 ± 0.01

I - Ionathan, GD – Golden Delicious, S – Starkrimson

Table 2. Chemical composition of the three apple varieties

	Chemical composition				
Variety	**Polyphenols (mg/ml galic acid)**	**Antioxidant capacity (% DPPH inhibited)**	**Total chlorophyll (µg/g)**	**Total carotene (µg/100 g)**	**Starch (mg/g)**
I	23.09 ± 0.59	4.21 ± 0.07	82.58 ± 0.00	702.48 ± 0.00	3.71 ± 0.31
GD	15.56 ± 0.12	4.13 ± 0.00	86.40 ± 19.00	275.13 ± 0.00	4.85 ± 0.27
S	65.74 ± 0.58	4.91 ± 0.07	15.30 ± 2.00	670.64 ± 0.00	4.88 ± 0.27

I - Ionathan, GD – Golden Delicious, S – Starkrimson

for "Granny Smith" apple variety, compared to the other varieties evaluated.

The content of total soluble differs depending on the variety of apple. Were recorded values between 19.25 and 23.25 °Brix. These values are consistent with values reported by Sestras *et al.* (2009).

Fruit pH varies depending on the variety, Ionathan variety registered lowest value in comparison with the other studied varieties. Our results are in agreement with those reported by Henriquez *et al.* (2010) who obtained similar values of pH.

In apples as well as in other fruits, the content of total phenolic compounds (TPC) varies depending on variety, harvest time and storage conditions. TPC large variation also occurs according to the apples color red, yellow, green, or bicolour skin but also because of the apples pulp which can be more or less bright and colorful (Drogoudi *et al.*, 2008). This can be seen in the case of our experiment, so Starkrimson variety had the highest content in TPC, followed by Golden Delicious variety and Ionathan (*Tab. 2*). Regarding antioxidant capacity there were no major differences between these varieties, having an average antioxidant capacity.

Chlorophyll content varies from one variety to another, as Golden Delicious had recorded the highest total chlorophyll content followed by Ionathan variety. Starkrimson variety recorded a slightly lower content (*Tab. 2*). Chlorophyll content varies depending on the variety and on the light intensity and exposure of fruit in the tree crown.

Carotenoid content was also different for each variety. Golden Delicious recorded the lowest levels of carotenoids, 275.13 µg/100 g. This was observed by Lizabethlister (1994) when for the Golden Delicious variety were recorded lower values compared to the other varieties studied. Changes in the levels of carotenoids are due to high intensity of light, temperature fluctuations and variety. These factors are well known for their

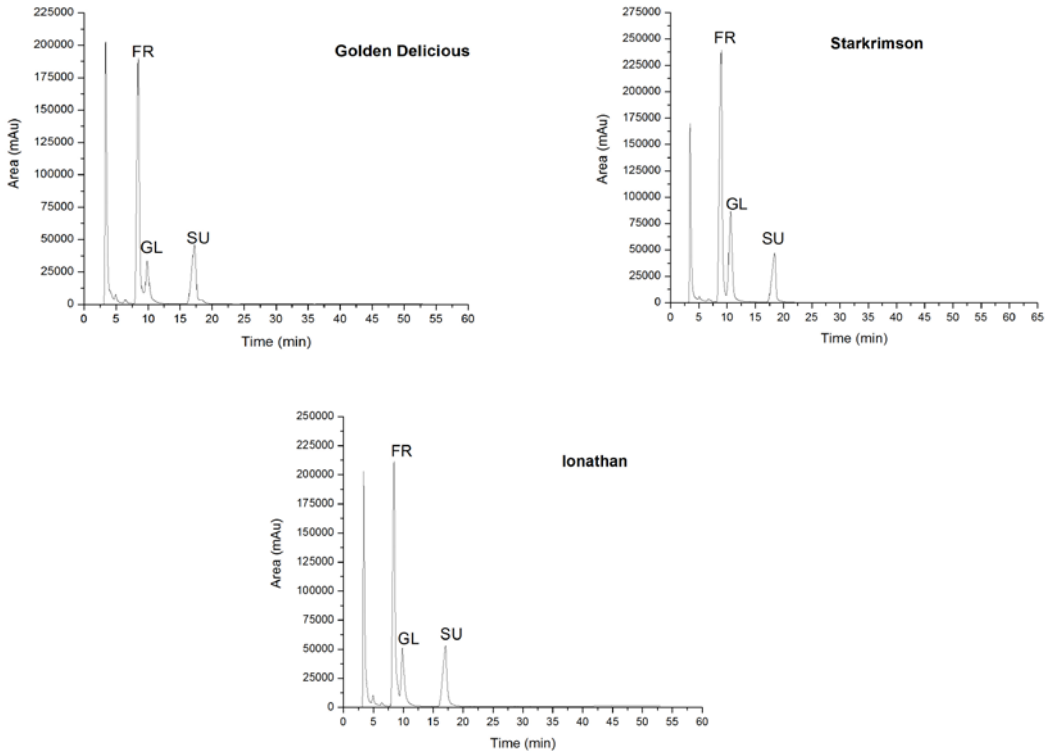

Fig. 1. Quantification of fructose (FR), glucose (GL) and sucrose (SU) from apples fruits (Ionathan, Golden Delicious and Starkrimson) by HPLC

influence on the degradation of pigments (Britton *et al.*, 1995).

The starch content is quite low for all three studied varieties because it has been converted into monosaccharides. Thus Ionathan variety recorded a value of 3.71 mg/g, the Starkrimson variety recorded 4.88 mg/g and Golden Delicious variety recorded 4.85 mg/g.

Regarding the content of sugars, fructose, glucose and sucrose were identified as the major monosaccharide found in fruits apple (*Fig. 1*).

Fructose level was always higher than glucose and sucrose (*Fig. 1*). This was observed by Jihong *et al.* (2007) for Delicious, Orin, Fuji and Ralls varieties. Fructose content varies depending on the variety, Starkrimson variety registered a content of 12.92 g/100 g juice, Ionathan variety recorded a 9.91g/100g juice and Golden Delicious variety recorded a content of 8.62 g/100g juice.

The content of glucose also varies for all three apple varieties, Starkrimson variety recorded a content of 4.01 g/100g juice, Ionathan variety registered a content of 2.00 g/100g and Golden Delicious registered a content of 1.49 g/100 g juice.

In terms of sucrose content, Ionathan variety gained 4.29 g/100g juice followed by Starkrimson variety (containing 3.77 g/100g juice) and Golden Delicious variety with a content of 3.49 g/100g juice. The levels of glucose, fructose and sucrose of the studied samples from this paper is in agreement with the ranges reported by Jihong *et al.* (2007).

CONCLUSION

Sugars profile of apple is an important component of the chemical composition for fruit quality assessment and provides valuable information about the authentication of apple juice.

Content in phenolic compounds is also one of the most important factors for the evaluation and characterization of apple varieties regarding their nutritional value and potential use for different products.

Following the results obtained, all three apple varieties are suitable for both fresh consumption and as compotes, juices or jams.

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