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ORIGINAL PAPER

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# ASSESSMENT OF PHYSICOCHEMICAL PROPERTIES OF AUTUMN AND WINTER APPLE VARIETIES AND THEIR PRETABILITY FOR JUICE EXTRACTION

Ioana Roman[1], Calin S. Vac[2], Raoul A. Roman[3]✉

[1] Department for Teacher Training, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3–5 Mănăștur, 400372, Cluj-Napoca, România

[2] Advanced Horticultural Research Institute of Transylvania, Faculty of Horticulture, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3–5 Mănăștur, 400372, Cluj-Napoca, România

[3] Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3–5 Mănăștur, 400372, Cluj-Napoca, România

## ABSTRACT

This study reveals data about the physicochemical properties of autumn and winter apple cultivars from Cluj-Napoca, Romania and technological transfer of the research data to apple breeding programs and nutrition area. The main objective pursued is materialized in the study of performances of five autumn-apple varieties and twelve winter ones regarding thirteen characteristics conferring high suitability for juice extraction. It also wants to formulate conclusions and recommendations for: consuming healthy foods and apple improvement programs in view of obtaining new cultivars of high quality fruits, suitable for fresh consumption as well as for juice production. Two of the oldest traditional Romanian apple varieties, 'Pătul' and 'Poinic', which have been chosen as references for the suitability of such cultivation for the production of juice, are famous for their juicy and balanced taste, riches in vitamin C and low in sugar content. It is obvious that for these traits there are large selecting possibilities of the genitors both as plus variants as well as minus variants, according to the direction of melioration being considered.

**Key words:** apple juice, characteristics, genitors, healthy food, technological transfer, triple helix

**Abreviations:** ANOVA – analysis of variance, GI – glycemic index, NPBTs – new plant breeding techniques

## INTRODUCTION

The apple, as we know it today, has an 8000-year-old wandering behind him. Starting from its Asian homeland, it began its triumphal march around the world. An exciting journey through the technological, practical and culinary aspects of the apple was presented by Tarjan [2006]. Apples are still a popular snack, owing to their high water and roughage sing compounds and their low-calorie count [Chadha and Awasthi 2005, Hecke et al. 2006]. The growing intake of apples and apple juice and their rich chemical composition suggest their important effect to increase the health of the populations consuming them. Apples are a rich source of nutrients, and they have antioxidant proprieties, so consumption of apples can reduce risk

✉ raoul.roman@gmail.com

of diabetes, some cancers, asthma, cardiovascular disease, and Alzheimer's disease. The findings obtained by Espley et al. [2014] demonstrate that "apple consumption affects aspects of inflammatory pathways and the gut microbiota".

Apples varies vary greatly in composition; also appear small changes in chemical composition during the maturation of the fruit [Slavin and Lloyd 2012]. A diet rich in fruit has health benefits, for apples these beneficial effects have been well documented [Hyson 2011, Yuan et al. 2011]. "Apple and apple products are one of the major dietary sources of antioxidants" [Espley and Martens 2013]. The range of apple varieties in the supermarkets has dramatically decreased. Only about 12 apple varieties, from integrated cultivation ('Jonagold', 'Jonathan', 'Starkrimson', 'Gala', 'Granny Smith') are sold all over Europe [Baker 2004]. In contrast to this small number, there are about 300 apple varieties from organically grown field sites ('Cox Orange', 'Gravensteiner'); most of these can only be bought at farmers' markets [Phillips 2005]. The old, natural orchard-grown varieties offer a wide range in flavour, aroma, sugar- and acid-content and phenolic compounds.

Rendering valuable – via processing – of plant raw-materials is a permanent aim of research in the field of horticulture, being generated by the necessity for a balanced range of foodstuffs all the year round [Beceanu et al. 2011]. Song et al. [2006] opined that little has been done in the area of apple melioration, to obtain varieties meant for processing; for such an aim, however, one encounters the tendency to imply varieties destined to consumption in fresh state, in the processing.

As for now, programs of apple research include objectives which refer to obtaining varieties for processing and identifying cultivars meeting the demands of processing entirely [Sanders 2010].

The main objectives of this research are to highlight the physicochemical properties and pretability for juice production of autumn and winter apple varieties, and follow three main traits, which interacted between them as a triple helix model (Fig. 1): studying the properties and availability of traditional Romanian apple varieties to create new hybrids; stimulating the population to consume healthy food because apple and apple juice have high vitamin C content and low sugar content; technological transfer over the apple breeding programs, nutritional programs and apple products' marketplace (juice, jam, compote, cakes etc.).

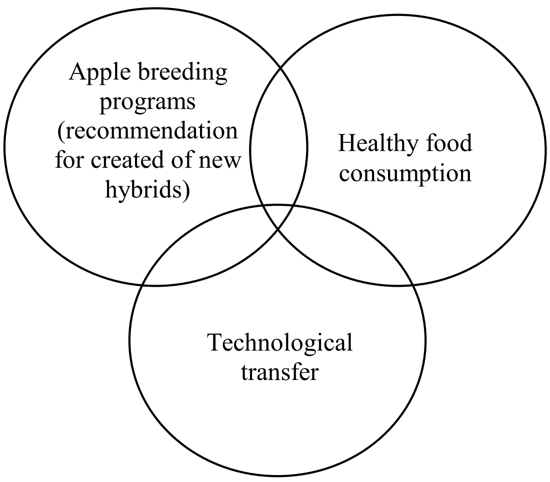


**Fig. 1.** Triple helix – impact of properties of autumn and winter apple varieties and pretability for juice production over the apple breeding programs, healthy food consumption and technological transfer

## MATERIALS AND METHODS

For this research the focus will be mainly on the triple helix concept. This concept "was recently introduced as an analytical framework that synthesizes the key features of Triple Helix interactions into an 'innovation system' format, defined according to the systems theory as a set of components, relationships and functions" [Ranga and Garzik 2015]. The triple helix perspective emphasizes on integration of: importance of apples' physicochemical properties for plant breeding programs, healthy food and technological transfer (Fig. 1). This also describes the interaction between these three aspects, followed by present research.

The biological material used in the experiments pertaining to this research has been represented by five autumn apple varieties and twelve of winter ones to be found in the variety collection of the University of Agricultural Science and the university's didactic farm Palocsay.

Sampling procedure – by every apple cultivar was taking 6 fruits (from different parts of trees 'crown').

For both autumn-apple varieties and the winter ones, testing has been carried out within comparative cultures set in blocks following a linear model, each including 15 to 25 trees. Thus, per total parcel-repeats, of each variant 36 to 60 trees were determined several physicochemical properties of the fruits: fruit yield (t/ha), dry-matter (%), juice yield (hl/ha), juice output (%), fruit weight (g), fruit volume ($cm^3$), fruit density ($g/cm^3$), sugar content (%), total acidity (%), vitamin C (%), juice density ($g/cm^3$), output index, losses (%). All analyses were carried out during 3 years.

For the traits expressed in metric units of measurement there were carried out three repeats/block, i.e., 3–5 trees/repeat, in view of computing and statistically interpret the results.

In order to compare the varieties of autumn and winter apples tested within the collection, the analysis of variance (ANOVA), i.e., the DL test has been used as both with the autumn and winter varieties of apples there exists the possibility to select controls of best defined characters, fit for juice production. Thus, for the cultivars of autumn apple the variety named 'Pătul' has been selected and, with the comparing culture for comparison with winter cultivars, the 'Poinic' variety has been selected. Both varieties are known for their low dry matter – percentage and obviously, a high water content that leads to a high juice-extraction output.

Determination of fruit yield was made by weighing, for each tree of the parcel-repetition. The results obtained were used for statistical interpretations of the series of variation and calculating the average parcel-repetition. Based on this final average, production per unit area (in t/ha), was calculated.

To determine dry matter content of fruit it was used the refractometric method. To achieve the analysis we used freshly squeezed apple juice from the fruit reached maturity [Fan et al. 2009].

Juice extraction was carried out with centrifugal extractor of 500 g fruit fresh/ experimental version. The quantity of juice resulting was measured and expressed in milliltre volume and then converted to hl/ha to assess the production of juice per unit area.

The methods for determination of weight, volume and density of fruit are described in "Manual of methods of analysis of foods. Fruits and vegetable products" [2016]. Fruit weight was determined by weighing on a balance of technical precision of each fruit and expressed the result in grams (g) with two decimal places. The individual measurements were used to calculate average fruit weight for each experimental variant. Fruit volume was determined from all the apples used for the analysis of each plot-repetition. Fruit density was calculated mathematically by dividing the fruit weight (g) to its volume ($cm^3$). Fruit density, determined at different times of harvesting, shows the level of maturation of apples.

Juice acidity percentage (estimated as malic acid equivalent) was determined by titration with NaOH and phenolphthalein indicator [Obeed et al. 2008]. For calculating total titratable acidity using the formula:

$$A\ (\%) = \frac{n \times f \times k \times v_1}{v_2} \times 100$$

where: n = amount of solution NaOH (ml) which has been titrated; f = solution factor 0.1 N NaOH (f = 0.312); k = acid factor corresponding to 0.1 ml N NaOH solution (k = 0.0067 g malic acid); $v_1$ = volume of extract before filtering (ml); g = weight of sample which was obtained extract (g); $v_2$ = volume of filtrate taken in the analysis (ml); A = titratable total acidity, expressed as % malic acid (predominant acid).

Vitamin C content (mg/100 g fresh fruit) was determined by volumetric method Shahnawaz at al. [2009]. The calculation result was based on the formula:

$$\text{Vitamin C (mg \%)} = \frac{n \times v_1 \times 0.352}{g \times v_2}$$

where: n = amount of solution 0,4 N $KIO_3$ (ml) used for titration; $v_1$ = volume subjected to titration ($v_1$ = 50 ml); $v_2$ = volume of filtrate (ml), ($v_2$ = 10 ml); g = mass of fruit taken in the analysis.

Juice output (%) was determined mathematically by comparing the quantity of juice extracted from fresh fruit to 100 kg fruit. The sugar content of fruit was expressed as a result, obtained by calculating the dry matter content (%). The formula used was as follows:

$$Z\ (\%) = \frac{n \times 4.25}{4} - 2.5$$

where "n" is the value for dry reading on the dial refractometer [Ardelean et al. 2006].

Output index is given by the ratio between mass and weight juice remains. Leftover (skin, seeds, pieces of pulp, stems, seminal lodges etc.) from each sample after the extraction of juice, were weighed in the balance technique to two decimal places. The value of output index can be in 2 and 5 range, it shows the ratio of plastic and mechanical elements of apples. For a correct interpretation of the output index it must be correlated with the volume of fruit juice and sugar content.

## RESULTS AND DISCUSSIONS

### Properties and availability of traditional Romanian apple varieties to create new hybrids (apple breeding programs)

The data from that three growing seasons, are highly to demonstrate how this research results contribute to a better understanding the processes related to the predisposition of the traditional apple cultivars for the processing as well as the apple juice quality ('Poinic' and 'Pătul'), regardless the vegetation season.

In the case of autumn apple varieties it was necessary to assess their suitability for juice production because these varieties have a very low shelf life and their water content is lost very quickly. Another reason was that one of the most popular autumn apple Romanian variety 'Pătul', famous for its high-content in juice, may be considered as control for comparing suitability, such as for cultivation, or for juice production.

Recorded values regarding the characters studied with the five autumn apple varieties tested at Cluj-Napoca over three years are displayed in (Tab. 1).

The data of (Tab. 1) show that the variety 'Pătul' (control) has registered the lowest fruit production per unit area, at the significant differences from all other varieties with which it was compared. Moreover, these differences are still very significant even when their meaning is expressed on the use variance interaction ($S^2_{V\times A}$). VThe explanation of such behaviour is that unlike the other varieties tested in comparative culture; 'Pătul' belongs to the group extensive variety cultivars (high growth vigour). Of the experimental point of view 'Pătul' deserves to be used as a control because of high water content of fruit and other characteristics that give an apple cultivar suitability for the production and extraction of juice.

In the dry matter content variety 'Pătul' (control) is ranked last, with only 11.48%. When comparison is based on the error variance ($S^2_E$) the other four varieties above very significantly exceed control ('Poinic') in the dry matter content. When comparison is based on interaction variance ($S^2_{V\times A}$), 'Florina' variety do not differ statistically from control in the dry matter content.

Data presented in Table 1, are proof that within the apple-variety collection at Cluj-Napoca there are to be found varieties of intensive type (such as 'Florina') displaying fruit productions of high level as to the area cultivated and dry matter – content comparable – or almost equal to that of 'Pătul' variety (control). Such varieties can be recommended for fruit production for juice extraction, on the one hand and – on the other, to be utilised as genitors in hybridization meant to elucidate the genetic determinism of such trait and to produce hybrid descendants of large variability of the respective trait, in which selection is able to act efficiently.

In his work, i.e., Mevlut [2005] obtained similar results with the 'Florina' variety having qualified it as highly productive and high succulence. 'Prima' and 'Ancuţa' varieties are as productive as 'Florina' but their adequacy of juice production is diminished by the much higher content of fruit in dry matter. Similar data on juice production were obtained from the POMOSANO project (Nr. 5-1a-238, CUP H21J12000060001), which showed that all fresh juices were produced using a standard method developed at the Laimburg experimental center [Stürz et al. 2015]. In their opinion the production of apple juice is defined as follows: "very low yield (average yield < 35%), low yield (35% ≤ average yield < 45%); average yield (45% ≤ average yield < 55%); good yield (55% ≤ average yield < 65%), very good yield (average yield ≥ 65%)" [Stürz et al. 2015].

One can conclude that juice production with surface unit is for sure influenced by productivity of the cultivar taken into consideration, on the one hand, as well as the output in juice of the respective cultivar's fruit yield, on the other.

The fact that none of the cultivars in trial proved any constancy in performance regarding the fruit weight all along the three years of experimenting, there suggests a low heritability of this trait, fact also noted by Herbinger et al. [2004].

**Table 1.** Traits of autumn-apple varieties conferring adequacy of juice production

| Variety | Fruit yield | Significance of difference | | Dry matter | Significance of difference | | Juice prod. | Significance of difference | |
|---|---|---|---|---|---|---|---|---|---|
| | t/ha | $S^2_E$ | $S^2_{V×A}$ | % | $S^2_E$ | $S^2_{V×A}$ | hl/ha | $S^2_E$ | $S^2_{V×A}$ |
| Ancuţa | 25.17 | xxx | xx | 12.33 | xxx | xx | 169.16 | xxx | xxx |
| Florina | 21.93 | xxx | – | 11.97 | xxx | – | 145.76 | xxx | xx |
| Generos | 21.86 | xxx | xxx | 13.47 | xxx | xxx | 139.65 | xxx | x |
| Pătul, Mt | 12.98 | – | – | 11.48 | – | – | 102.40 | – | – |
| Prima | 20.68 | xxx | xx | 12.34 | xxx | xx | 146.77 | xxx | xx |
| DL5% | | 0.98 | 1.96 | | 0.03 | 0.58 | | 20.0 | 26.4 |
| DL1% | | 1.33 | 2.85 | | 0.04 | 0.84 | | 27.1 | 38.4 |
| DL0.1% | | 1.78 | 4.28 | | 0.06 | 1.26 | | 36.3 | 54.6 |

| Variety | Fruit weight | Significance of difference | | Fruit volume | Significance of difference | | Fruit density | Significance of difference | |
|---|---|---|---|---|---|---|---|---|---|
| | g | $S^2_E$ | $S^2_{V×A}$ | $cm^3$ | $S^2_E$ | $S^2_{V×A}$ | $g/cm^3$ | $S^2_E$ | $S^2_{V×A}$ |
| Ancuța | 184.67 | xxx | – | 230.11 | xxx | – | 0.80 | xx | x |
| Florina | 180.11 | xxx | – | 240.78 | xxx | – | 0.75 | – | – |
| Generos | 147.44 | xx | – | 172.44 | – | – | 0.85 | xxx | xxx |
| Pătul, Mt | 120.44 | – | – | 158.56 | – | – | 0.76 | – | – |
| Prima | 124.89 | – | – | 156.56 | – | – | 0.80 | xx | x |
| DL5% | | 18.36 | 64.43 | | 30.59 | 84.14 | | 0.03 | 0.04 |
| DL1% | | 24.95 | 93.71 | | 41.57 | 122.39 | | 0.04 | 0.06 |
| DL0.1% | | 33.42 | 140.56 | | 55.68 | 183.58 | | 0.06 | 0.07 |

xxx – very significantly positive, xx – distinct significantly possitive, x – less significantly positive,
– no statistically differences

The highest volume of fruit (Tab. 1) was recorded with 'Florina' and 'Ancuța'; with these, the differences vis-á-vis control – are very significantly positive on comparing to error variance ($S^2_E$). It is obvious that in what fruit volume is concerned; genotypes differ among themselves, with differences statistically granted. The variants tested differ utterly in what fruit density is concerned, trait pertinent to establishing the optimal timing of directing the fruits into processing. It has been taken into account that from the point-of-view of weight, volume, density, sugar content of the fruits, the collection under study contains autumn varieties successfully fit to obtain juice and concentrates of high quality (such for instance 'Generos', 'Ancuța', and 'Florina').

Fruit acidity is an essential component, which was reflected in the quality of apple juice (taste, storage, etc.). The results about the total acidity indicate that in the autumn apple collection is possible to identify genitors containing moderate acidity of the fruit. Such are the favourite fruit of juice production, conferring them balanced sweet-sour flavour taste specifically the apple juice.

The results thus obtained (Tab. 2) is the proof that in the studied autumn apple variants in the collection there can discover an obvious variability in the overall acidity and C vitamin content of fruits, that allows for an effective selection (as genitor or for melioration purpose), in any of the desired directions. Similar studies regarding C-vitamin content of apple fruits were carried out by Espley et al. [2014] the latter even noticed substantial alterations in the C-vitamin content from one year to another, within the same variant.

Similar values regarding the characteristics of apple juice are also displayed in Commission Directive relating to fruit juices and certain similar products intended for human consumption.

Fruits that are high in vitamin C and sugar are preferred for obtaining apple concentrate, or their juice can be diluted to the desired concentration of the consumer. If the juice is added exogenous Vitamin C, this must be declared in the ingredients list [Official Journal of the European Union 2011].

Loss assessment is rather important to the economy of the technological process of extracting juice, as owing to loss reduction one can increase extraction efficiency.

Following the DL-test, it is noticeable that the values in the juice-efficiency differences, when comparing on the basis of error variance ($S^2_E$) and on that of the interaction variance ($S^2_{V\times A}$), of the four variants taken into study are negative and very significant as to the juice output of 'Pătul' (control). The juice-output values are comprised between 78.9% with 'Pătul' (control) and 63.8% with 'Generos' (Tab. 2). Similar results were also obtained by Mevlut [2005]. For 'Pătul', the juice output obtained by this author was of 72.5%. Output index was computed, most frequently, with the residue obtained by pressing apples as such feature has been studied, to a lesser extent, with apple juice.

**Table 2.** Traits of autumn-apple varieties conferring adequacy for a healthy food and for juice production

| Variety | Total acidity | Significance of difference | | Vitamine C | Significance of difference | | Losses | Significance of difference | |
|---|---|---|---|---|---|---|---|---|---|
| | % | $S^2_E$ | $S^2_{V\times A}$ | mg | $S^2_E$ | $S^2_{V\times A}$ | % | $S^2_E$ | $S^2_{V\times A}$ |
| Ancuţa | 0.511 | xxx | xxx | 10.07 | xxx | xxx | 2.33 | xxx | – |
| Florina | 0.139 | ooo | – | 5.45 | oo | – | 2.27 | xxx | – |
| Generos | 0.465 | xxx | xx | 10.97 | xxx | xxx | 2.41 | xxx | – |
| Pătul, Mt | 0.174 | – | – | 5.77 | – | – | 1.39 | – | – |
| Prima | 0.124 | ooo | – | 4.39 | ooo | – | 1.27 | – | – |
| DL5% | | 0.14 | 1.55 | | 0.19 | 1.71 | | 0.36 | 1.84 |
| DL1% | | 0.20 | 2.25 | | 0.26 | 2.48 | | 0.49 | 2.67 |
| DL0.1% | | 0.26 | 3.21 | | 0.34 | 3.53 | | 0.66 | 4.01 |

| Variety | Juice output | Significance of difference | | Sugar content | Significance of difference | | Juice density | Significance of difference | | Output index | Significance of difference | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | $S^2_E$ | $S^2_{V\times A}$ | % | $S^2_E$ | $S^2_{V\times A}$ | g/cm$^3$ | $S^2_E$ | $S^2_{V\times A}$ | | $S^2_E$ | $S^2_{V\times A}$ |
| Ancuţa | 67.2 | ooo | ooo | 8.20 | xxx | xxx | 1.079 | xx | x | 2.30 | ooo | ooo |
| Florina | 66.5 | ooo | ooo | 3.29 | – | – | 1.040 | – | – | 2.25 | ooo | ooo |
| Generos | 63.8 | ooo | ooo | 9.16 | xxx | xxx | 1.092 | xxx | xxx | 2.49 | ooo | ooo |
| Pătul, Mt | 78.9 | – | – | 3.63 | – | – | 1.024 | – | – | 3.98 | – | – |
| Prima | 70.9 | ooo | ooo | 2.17 | ooo | – | 1.041 | xx | x | 2.59 | ooo | ooo |
| DL5% | | 0.85 | 3.86 | | 0.49 | 1.86 | | 0.03 | 0.04 | | 0.04 | 0.33 |
| DL1% | | 1.16 | 5.61 | | 0.67 | 2.71 | | 0.04 | 0.06 | | 0.05 | 0.48 |
| DL0.1% | | 1.55 | 7.99 | | 0.89 | 3.85 | | 0.06 | 0.07 | | 0.07 | 0.72 |

xxx – very significantly positive, xx – distinct significantly possitive, x – less significantly positive, ooo – very significantly negative, oo – distinct significantly negtive,
– no statistically differences

**Table 3.** Traits of winter-apple varieties conferring adequacy for juice production

| Variety | Fruit yield | Significance of difference | | Dry matter | Significance of difference | | Juice prod. | Significance of difference | |
|---|---|---|---|---|---|---|---|---|---|
| | t/ha | $S^2_E$ | $S^2_{V\times A}$ | % | $S^2_E$ | $S^2_{V\times A}$ | hl/ha | $S^2_E$ | $S^2_{V\times A}$ |
| Goldspur | 26.43 | xxx | xxx | 14.27 | xxx | – | 185.02 | xxx | xxx |
| Granny Smith | 16.03 | – | – | 13.86 | xxx | – | 100.43 | – | – |
| Gustav Durabil | 24.63 | xxx | xxx | 14.16 | xxx | – | 156.53 | xxx | x |
| Idared | 27.00 | xxx | xxx | 12.70 | xxx | – | 176.01 | xxx | xx |
| Jonagold | 27.77 | xxx | xxx | 12.47 | xxx | – | 195.07 | xxx | xxx |
| Jonathan | 21.83 | xxx | xx | 12.39 | xxx | – | 158.62 | xxx | xx |
| Pinova | 25.47 | xxx | xxx | 12.81 | xxx | – | 173.52 | xxx | xx |
| Poinic, Mt | 14.27 | – | – | 11.71 | – | – | 108.70 | – | – |
| Starkrimson | 25.73 | xxx | xxx | 13.16 | xxx | – | 172.62 | xxx | xx |
| Topaz | 20.50 | xxx | x | 13.98 | xxx | – | 130.43 | x | – |
| Wagener Premiat | 26.23 | xxx | xxx | 13.62 | xxx | – | 161.36 | xxx | xx |
| Golden Reinders | 28.53 | xxx | xxx | 12.67 | xxx | – | 185.62 | xxx | xxx |
| DL5% | | 2.50 | 4.51 | | 0.40 | 2.77 | | 17.52 | 34.10 |
| DL1% | | 3.32 | 6.56 | | 0.53 | 4.03 | | 23.27 | 49.60 |
| DL0.1% | | 4.28 | 9.33 | | 0.68 | 5.74 | | 29.99 | 70.56 |

| Variety | Fruit weight | Significance of difference | | Fruit volume | Significance of difference | | Fruit density | Significance of difference | |
|---|---|---|---|---|---|---|---|---|---|
| | g | $S^2_E$ | $S^2_{V\times A}$ | $cm^3$ | $S^2_E$ | $S^2_{V\times A}$ | $g/cm^3$ | $S^2_E$ | $S^2_{V\times A}$ |
| Goldspur | 136.06 | – | – | 166.33 | – | – | 0.797 | – | – |
| Granny Smith | 215.33 | xxx | x | 241.78 | xxx | x | 0.807 | – | – |
| Gustav Durabil | 180.22 | xxx | – | 212.00 | xxx | – | 0.850 | – | – |
| Idared | 254.56 | xxx | xx | 325.78 | xxx | xx | 0.787 | – | – |
| Jonagold | 222.89 | xxx | x | 275.33 | xxx | x | 0.811 | – | – |
| Jonathan | 137.11 | – | – | 169.11 | – | – | 0.811 | – | – |
| Pinova | 160.56 | xxx | – | 188.67 | xx | – | 0.824 | – | – |
| Poinic, Mt | 122.44 | – | – | 149.56 | – | – | 0.820 | – | – |
| Starkrimson | 177.22 | xxx | – | 213.78 | xxx | – | 0.829 | – | – |
| Topaz | 179.00 | xxx | – | 201.89 | xxx | – | 0.848 | – | – |
| Wagener Premiat | 141.33 | – | – | 162.89 | – | – | 0.866 | x | – |
| Golden Reinders | 151.00 | xx | – | 185.11 | xx | – | 0.814 | – | – |
| DL5% | | 21.24 | 75.08 | | 24.58 | 89.08 | | 0.040 | 0.062 |
| DL1% | | 28.22 | 109.21 | | 32.65 | 129.58 | | 0.056 | 0.079 |
| DL0.1% | | 36.37 | 155.37 | | 42.09 | 184.34 | | 0.072 | 0.093 |

xxx – very significantly positive, xx – distinct significantly positive, x – less significantly positive,
– no statistically didderences

It is noticeable that the highest value in the output index is obtainable with 'Pătul' (control).

In the case of winter apple varieties it was necessary to assess their suitability for juice production because these varieties are usually used by food industry. Winter varieties are resistant to transport and storage much better than autumn varieties, and maintain their water content and gustative qualities. It must also be kept in mind that winter varieties occupy an area much larger than the autumn ones. In this way, they constitute a large source of raw material for juice extraction, source much richer and more lasting.

One of the most popular winter apple Romanian variety, 'Poinic', was chosen as control for comparison suitability as such a cultivation is fit for the production of juice, the more so as it is famous for its high juicy quality and sweet taste, and well balanced, too.

Data regarding the traits analysed with the twelve winter-apple varieties under study are introduced by Table 3. The obtained results seem to point to an accentuated dependence of fruit production, juice production and dry matter content on the special conditions of each of the experimentation years and a relatively shallow genetic determination. The data presented in Table 3, show that the 'Poinic' variety (control) has the lowest production of fruit per unit area. Variety 'Poinic' has registered the significant differences from most other varieties when comparison is based on the error variance ($S_E^2$). 'Granny Smith' was the only variety in which the difference from the control based on error variance ($S_E^2$) was insignificant. 'Granny Smith' is an old variety, semi-intensive, very similar to 'Poinic' variety.

In his study, Tarjan [2006] obtains similar results in the juice production (hl/ha) of some winter-apple varieties such as 'Goldspur' and 'Idared' characterised by them as very productive and of high succulence. From this point-of-view it is possible to assert that the results obtained by us are on line with literature data. Despite of its remarkable juiciness, 'Poinic' variety (control) has very low fruit yield (hl/ha). The fact that so few varieties manage to maintain their mean fruit weight along the three years of experimenting (see the significance of differences based on interaction variance), there suggests that this trait is strongly influenced by the conditions of the natural and culture technology environment. As with fruit weight, its volume is also strongly influenced by the conditions of the natural and man-made culture conditions, influences that more often than not, hide the contribution of the genotype to the phenotypical expression of the respective trait [Ardelean et al. 2006].

Fruit density reveals its consumer's maturation degree and knowing its value is important too, both for apple's industrial processing as well as for its storage. Depending on fruit density (g/cm$^3$), data in Table 3 only reveals the 'Wagener Premiat' variety with which the difference to the control is significant on comparison based on error variances ($S_E^2$).

When comparing based on interaction variance ($S_{V \times A}^2$), all the tested varieties are statistically equal to the control. The fact that not a one variant exceeds control in all three experimenting years, there suggests the almost entire dependence of this trait on environmental conditions, within a polygenic genetic determinism where, most probably, the effects of epistasy prevail.

The results hint of the fact that the collection of winter apple studied contains cultivars that have the genetic determinism of total acidity extremely obvious. In the case of apple, total acidity is controlled by two genetic models: one monogenic for the mean acidity and high/low and one polygenic, for the total acidity.

The C-vitamin content (Tab. 4) of fruit with the twelve winter-apple varieties analysed has displayed very high variability (2.75–17.75 mg/100 g fresh fruit). In the study conducted by Bassi et al. [2017], they found 14 cultivars with witamin C (ascorbic acid) levels higher than 3.0 mg/L; concentrations ranged from 0.74 ('Scilate') to 7.50 mg/L ('LUB A11706'). Varming et al. [2013], also reported value of vitamin C between 3.1–10 mg/L in freshly pressed juices of old Danish apple cultivars. The content in vitamin C of apple juice is important for antioxidant properties. It is known that vitamin C content is higher in the peel than in apple juice; their study Liu et al. [2018], demonstrated that vitamin C content ranges from 2.68 to 88.95 mg 100 g$^{-1}$ in fresh apple peels. The antioxidant effects of vitamin C from juice fruit was revealed by Alvarez-Parrilla et al. [2010].

It was observed that fruit presents different effects such variability suggests right from the very beginning, a strong dependence of the trait from the genotype of each cultivar.

**Table 4.** Traits of winter-apple varieties conferring adequacy for a healthy food and for juice production

| Variety | Total acidity | Significance of difference | | Vitamin C | Significance of difference | | Losses | Significance of difference | |
|---|---|---|---|---|---|---|---|---|---|
| | % | $S^2_E$ | $S^2_{V×A}$ | mg | $S^2_E$ | $S^2_{V×A}$ | % | $S^2_E$ | $S^2_{V×A}$ |
| Goldspur | 0.276 | xx | – | 7.40 | xxx | – | 1.441 | x | – |
| Granny Smith | 0.531 | xxx | xx | 8.65 | xxx | – | 2.258 | xxx | x |
| Gustav Durabil | 0.335 | xxx | – | 8.34 | xxx | – | 2.522 | xxx | x |
| Idared | 0.280 | xx | – | 2.75 | ooo | o | 1.813 | xxx | – |
| Jonagold | 0.346 | xxx | – | 3.80 | ooo | o | 1.587 | xx | – |
| Jonathan | 0.533 | xxx | xx | 9.00 | xxx | – | 1.053 | – | – |
| Pinova | 0.194 | – | – | 4.00 | ooo | – | 1.489 | x | – |
| Poinic, Mt | 0.137 | – | – | 6.76 | – | – | 0.960 | – | – |
| Starkrimson | 0.182 | – | – | 2.86 | ooo | o | 1.891 | xxx | – |
| Topaz | 0.668 | xxx | xxx | 8.56 | xxx | – | 2.596 | xxx | x |
| Wagener Premiat | 0.498 | xxx | xx | 17.75 | xxx | xxx | 2.244 | xxx | x |
| Golden Reinders | 0.290 | xxx | – | 4.57 | ooo | – | 1.287 | – | – |
| DL5% | | 0.087 | 0.237 | | 0.29 | 2.82 | | 0.467 | 1.250 |
| DL1% | | 0.115 | 0.344 | | 0.39 | 5.92 | | 0.621 | 1.818 |
| DL0.1% | | 0.149 | 0.490 | | 0.50 | 9.12 | | 0.800 | 2.587 |

| Variety | Juice output | Significance of difference | | Sugar content | Significance of difference | | Juice density | Significance of difference | | Output index | Significance of difference | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | $S^2_E$ | $S^2_{V×A}$ | % | $S^2_E$ | $S^2_{V×A}$ | g/cm$^3$ | $S^2_E$ | $S^2_{V×A}$ | | $S^2_E$ | $S^2_{V×A}$ |
| Golden Delicious | 69.99 | ooo | o | 12.66 | xxx | x | 1.027 | x | – | 2.516 | ooo | oo |
| Granny Smith | 62.71 | ooo | ooo | 12.22 | xxx | x | 1.051 | xxx | xx | 2.072 | ooo | ooo |
| Gustav Durabil | 63.56 | ooo | ooo | 12.54 | xxx | x | 1.063 | xxx | xxx | 2.063 | ooo | ooo |
| Idared | 65.17 | ooo | ooo | 10.99 | xxx | – | 1.029 | xx | – | 2.124 | ooo | ooo |
| Jonagold | 70.28 | ooo | o | 10.75 | xxx | – | 1.027 | x | – | 2.485 | ooo | oo |
| Jonathan | 72.66 | ooo | – | 10.66 | xxx | – | 1.038 | xxx | x | 2.665 | ooo | o |
| Pinova | 68.18 | ooo | oo | 11.11 | xxx | – | 1.034 | xxx | – | 2.326 | ooo | oo |
| Poinic, Mt | 76.16 | – | – | 9.94 | – | – | 1.019 | – | – | 3.065 | – | – |
| Starkrimson | 66.91 | ooo | oo | 11.48 | xxx | – | 1.035 | xxx | – | 2.273 | ooo | ooo |
| Topaz | 63.18 | ooo | ooo | 12.35 | xxx | x | 1.057 | xxx | xx | 2.065 | ooo | ooo |
| Wagener Premiat | 61.50 | ooo | ooo | 11.97 | xxx | – | 1.052 | xxx | xx | 1.933 | ooo | ooo |
| Golden Reinders | 65.04 | ooo | ooo | 10.96 | xxx | – | 1.033 | xxx | – | 2.075 | ooo | ooo |
| DL5% | | 1.06 | 5.04 | | 0.14 | 2.04 | | 0.008 | 0.020 | | 0.104 | 0.372 |
| DL1% | | 1.41 | 7.33 | | 0.19 | 2.97 | | 0.011 | 0.029 | | 0.138 | 0.542 |
| DL0.1% | | 1.82 | 10.43 | | 0.24 | 4.22 | | 0.014 | 0.042 | | 0.178 | 0.771 |

xxx – very significantly, xxx – very significantly positive, xx – distinct significantly possitive, x – less significantly positive, ooo – very significantly negative, oo – distinct significantly negtive, o – less significantly negative,
– no statistically differences"

Similar results regarding the content in titratable acidity and juice output were obtained by Juranovic [2011] and Hoehn et al. [2003]. In view of a correct interpretation of the output index, it has to be correlated with the juice volume and its content in sugars [Tarjan 2006]. The higher the concentration of sugars in the apple juice, the higher its weight will be. That's why the varieties renown as having high potential of sugar accumulation – such as 'Goldspur' that has an output index of 2.5 is situated – from this point-of-view – not far from cultivars 'Poinic' (3.065) and 'Jonathan' (2.665), varieties with juicy pulp but lower potential in sugar accumulation. The data analysed seem to suggest that this trait possesses a powerful genetic determinism, being given the significance of differences analysed on the basis of the interaction variance ($S^2_{V \times A}$) statistically granted from significant to very significant for all variants. The low value for sugar content ('Idared' 2.75%) conduct to low value for glycemic index, which suggest a lower risk of type 2 diabetes associated with the consumption of apple juice obtain by this variety of apple. In her study Hyson [2011] reveals that recent data have suggested a possible link between consumption of apple juice and reduced diabetes' risk.

The results obtained by present study are complementary with a research realized by Farneti et al. [2015] which reveals that "comprehensive screening will assist in the selection of Malus accessions with specific nutraceutical traits suitable to establish innovative breeding strategies or to patent new functional foods and beverages".

**Health benefices of apples and apple juice consumption** were highlighted by the high content of vitamin C – 'Generos' 10.97 mg and 'Wagener Premiat' 17.75 mg (Fig. 2). These varieties of apple are rich sources of citamin C that function as antioxidant and have an important role in disease prevention and increase the human body's immunity through protective mechanisms. Apples are recommended as a source of vitamin C and other antioxidants like: polyphenolics compounds, malic acid, vitamin $B_1$, $B_2$ [Slavin and Lloyd 2012]. Antioxidants' ability of deactivating free radicals has been used to treatment some pathologies as cancer and cardiovascular diseases [Scafuri et al. 2016]. Also the similar data about the nutritional physiology and health relevant characteristics of apples, the pomological, chemical and sensory information regarding the various apple varieties and their juices were described in the POMOSANO project at the Laimburg Research Centre [Stürz at al. 2015].

Thus, most of the apples consumed are processed, frozen, canned, or dried; all these processes determine degradation of antioxidant capacity of fruits. Consumption of fresh apple juice is healthy while consumption of juice with added sugar is harmful to the human body. Higher consumption of sugar sweetened fruit juice was associated with a 7% greater incidence of type 2 diabetes [Imamura et al. 2015]. Sugar content varies greatly at autumn apple cultivars compared to winter apples; this aspect can be seen in Figure 3. For autumn apples varieties the sugar content was very low ('Prima' 2.17%, 'Florina' 3.29% and 'Pătul' Mt. 3.63%). In the case of winter varieties, the sugar content was higher, ranging from 9.94% 'Pătul' to 12.66% 'Golden Delicious'. The data of this research shows that apples and apple juice are recommended for consumption because they have a low sugar content compared to other fruits. Also according to Atkinson et al. [2008], the glycemic index (GI) of apples is 36 ±2, lower than orange, which has 43 ±3 GI; and GI for apple juice is 41 ±2 compared to orange juice, for which the GI is 50 ±2. These aspects come to emphasize once again the importance, for human health, of apples and apple juice consumption because they have low sugar content and low glycemic index.

**Technological transfer** is the next mandatory step, because data obtained through this research should be applied by the apple breeding programs, nutrition programs and apple products' marketplace accordingly to the real societal needs. It is desirable to introduce hybrids, apple varieties, research results into the economic circuit, research results, in order to increase the efficiency and quality of products, processes, or the acquisition of new products, which are demanded on the market, or by adopting innovative behaviour, thus transferring products or technologies from the research stage to the market. This study highlights the importance of introducing varieties with valuable physicochemical properties and high productivity in apple orchards.

This will be an economic growth for both small and large apple growers; the introduction of valuable varieties of apple into plant breeding programs will also lead to new varieties/hybrids with better properties.

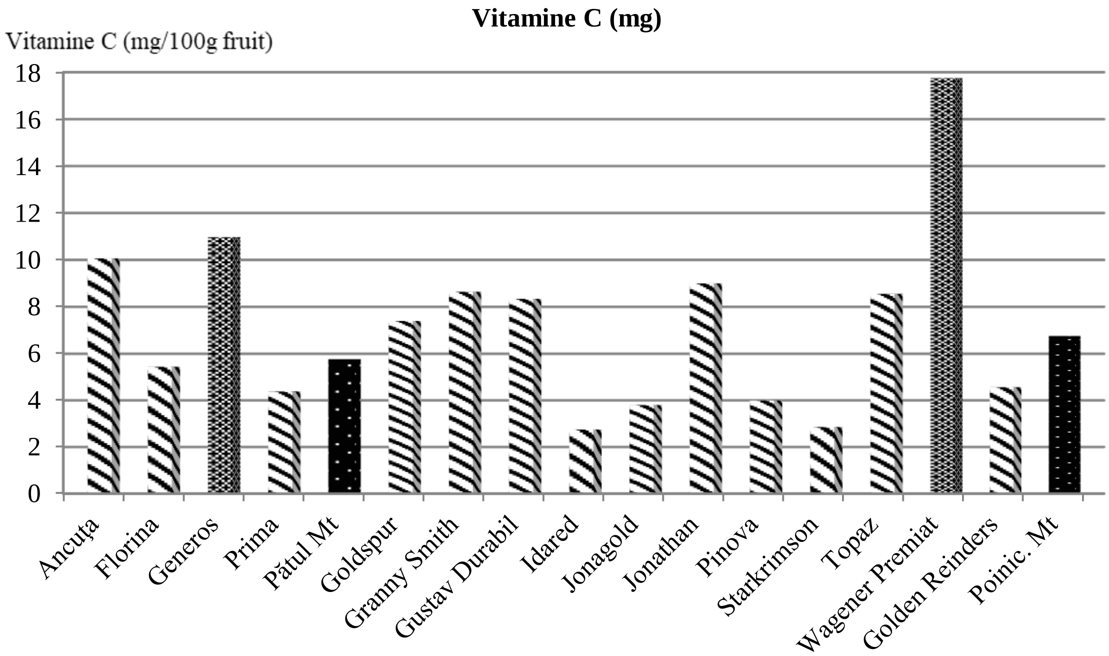


**Fig. 2.** Content of Vitamin C (mg/100 g fruit) – autumn and winter apple variety

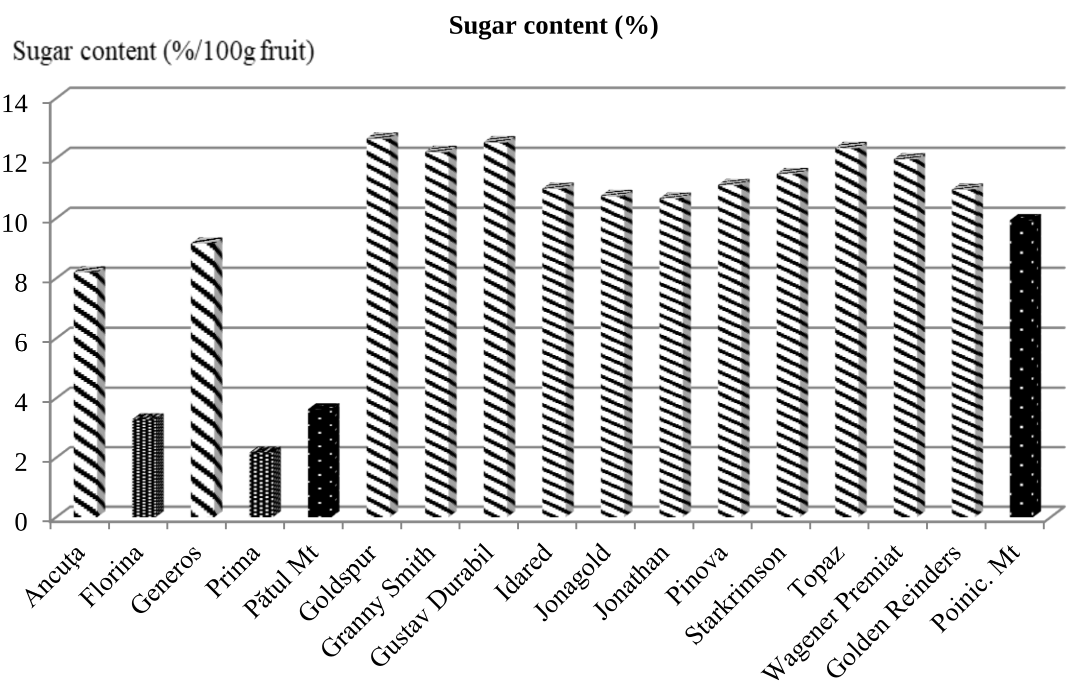


**Fig. 3.** Sugar content (%) – autumn and winter apple variety

At the same time, the traditional Romanian apples cultivars 'Poinic', 'Pătul' are valuable physicochemical properties and high resistant to dizzies and pets, in this case they should be valorous as genitors. "Green' biotechnologies" evidenced by the new plant breeding techniques (NPBTs) potentially allow to obtain apple tree with enhanced qualitative traits or pathogen-resistant by introducing specific genes and by means of minimal modifications in the apple genome. The genetic improvement of traditional autumn and winter Romanian apple cultivars may gain a great benefit from the new breeding techniques, because they do not alter the genetic heritage of the cultivar. Apples are the one of most important fruit crop in the Romania country an economical point of view, so it is a particular case for NPBTs because of traditional aspects, variety of cultivar, and consumer demands, which conducted to use of breeding techniques and furthermore, the application of genetic engineering to apple cultivars.

In opinion of Sedov [2014] "it may be necessary to revise the range of already existing apple cultivars and directions in breeding".

Consumptions of apple and apple juice (obtain by cultivars like: 'Prima', 'Pătul', 'Poinic', 'Idared', 'Jonathan', 'Jonagold' and 'Golden Reinders'), with low sugar content and high nutrient content can improve health and weight of children or adults. It's also known that children prefer to consume a lot of juice, in this situation apple juice with low sugar content should contribute to child obesity prevention.

The launch of a new product like 100% natural apple juice with low calorie and high nutrient contents on market which is quickly developing, needs a marketing plan. The product can be obtain from traditional variety of apple/hybrids, especially designed for the consumers, who are increasingly interested in fresh and healthy products.

Mass production of apples has been reduced to our country, because lot apple orchards were decommissioned. Population has to retain economic growth with new activities through the industry, agriculture or service sector. It is necessary to prevent this issue by create new orchards with apple varieties whose properties are enriched. It will be possible utilizing new hybrids obtained by traditional Romanian apple cultivars, which are resistant to pests or disease and have rich nutrient content.

## CONCLUSIONS

The results discussed conduct to an obvious clue that the collections of autumn and winter apple varieties studied display the possibility of identifying cultivars of high and very high yields per surface unit, as well as of high juiciness. These might be used mainly for juice production or, might be implied as genitors in apple-melioration programmes.

Of the traits analysed with both autumn- and winter apples, the widest variability has been displayed by fruit yield/unit of surface (t/ha); juice yield/surface unit (hl/ha); total acidity (%), and vitamin C-content (mg/100 g fresh fruit).

The winter apple varieties are more precious than the autumn ones in what the traits conferring on them the fitness to juice extraction are concerned. Against all odds, there exist precious autumn varieties such as 'Florina', uniting several fruit characteristics that confer suitability for juice extraction thus recommending it as possible genitor.

When it was taken into account that most of the raw material for apple-juice production is represented by the production of unsaleable dessert fruit, it is obvious that in selecting the genitors for a fair suitability to juice extraction, the traits granting the apples the high quality as dessert fruit, should also be taken into consideration.

Selecting genitors for suitability to juice production only on basis of the analysed traits could be totally mistaken when complimentary data able to reveal the correlations among these traits and their effect upon juice output are missing.

With the two apple collections studied there exists the possibility of nominating certain genitors that, generally, sum up as many of the fruit traits highest in rank of suitability for juice production. Variability of fruit traits conferring suitability to juice production is obviously larger for winter apples than in the autumn ones.

The future studies should be made on the creation of new varieties/ hybrids of apple trees that have genitors' properties, a balanced taste, low sugar content and rich Vitamin C, to ensure a healthy lifestyle and nutrition for the consumers and to meet their expectations. Also, by cultivating apple varieties with high productivity, succulent and valuable physicochemical properties, a higher profit/ a growth rate will be recorded for apple growers.

In the future research, should be important to explore the technical challenges which may increase the application of NPBTs to traditional Romanian apple cultivars, in particular focusing on the gene transfer (genes which induce low sugar content, high vitamin C content and resistance to diseases and pests).

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## AUTHOR CONTRIBUTIONS

Ioana Roman designed experimental activities and performed measurements. Calin Vac has investigated the impact on technological transfer of research data. Alin Raoul Roman performed statistical analysis. All authors equally contributed to manuscript writing and have equal rights.

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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