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Comparative analysis of Hungarian commercial apple juices

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SUMMARY

In recent years, 100% fruit juices have become increasingly popular among consumers. Beverages made from apples include a wide range of products, interest being particularly grate in direct press juices and products of organic farming that are produced "chemical-free". Our objective is to compare 100% apple juices produced using different technologies, which are commercially available in Hungary, by the complex analysis of some of their quality parameters.

For the tests, 12 varieties of 100% apple juices were used. The patulin contamination of the products was checked with the help of the Food and Feed Safety Directorate of the National Food Chain Safety Office by means of an HPLC-UV measurement following a special clean-up. The hydroxymethylfurfural content of the products were determined by spectrophotometry, antioxidant capacity by FRAP (Ferric Reduction Antioxidant Power), CUPRAC (Cupric Ion Reducing Antioxidant Capacity), DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) methods based on radical neutralization, and the total polyphenol content by the Folin-Ciocalteu method. Total dry matter content and ash content were determined by gravimetry, while the total soluble dry matter content was determined by refractometry. For the evaluation of the results, descriptive statistical analyses, variance analysis, non-parametric statistical tests, Spearman rank correlation and a statistical method based on sum of rank differences (SRD) multicriteria comparison were used.

The patulin contamination did not reach the maximum legal limit value for any of the products. Significant differences were found between the total and soluble dry matter contents of the various product groups. The ash content varies, but there is no significant difference between the two product categories. In general, the hydroxymethylfurfural content is higher in the case of products manufactured from concentrate, however, it was a press juice that contained extreme amounts of this compound. In the case of press juices, higher antioxidant activities and polyphenolic compound contents were found by all method. There are significant differences between the individual products in terms of the latter parameters ($p<0.05$). Using the SRD method, it was determined which of the products tested possessed the most optimal characteristics from a nutrition science point of view.

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The product groups tested differ significantly from each other in terms of almost all of the parameters measured. In general, the dry matter content of the press juices is higher, as a result of the fiber content. These products have higher antioxidant capacity values and polyphenolic compound contents, so they are nutritionally more beneficial. The latter parameters correlate well with each other, indicating the dominance of polyphenols in the development of antioxidant properties.

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Introduction

Ethnobotanical characteristics of apple

Today's well-known apple *(Malus×domestica* Borkh.*)* is a species that belongs to the *Rosaceae* family and the *Maloideae* subfamily. Its origin is unclear, it has emerged from the cross-breading of several apple species indigenous to Europe and Asia **[1]**. From the point of view of climatic conditions, it prefers moderately cool and rainy areas and mild winters, thus it exhibits a widespread geographical distribution. For dietary purposes, the false fruit of the plant is used, which is typically round in shape, with its color differing by variety. The fruit can be of a single color, yellow, green or red, or the combination of these, which is called complex coloring. The flesh of the fruit is surrounded by a waxy skin. It is a widely used raw material for the manufacture of more than 200 kinds of products **[2]**.

Apple production in Hungary

In Hungary, based on 2016 data, apples were produced on 34,704 hectares **[3]**, 588 hectares of which performed organic farming **[4]**. The quantity produced was 497,108 tons, 62,663 tons of which were put to industrial use, i.e., apple products, such as unfiltered and filtered apple juice, as well as apple juice concentrate were manufactured, among other things **[3]**.

Nutritional parameters of apples

The nutritional parameters of apples differ by variety, but in general it can be said that their energy content is low, only 31-52 kcal per 100 grams. Their carbohydrate content is 7-14 g/100 g, most of which is fructose. The fiber content is approximately 2 g per 100 grams, 70% of which is water-insoluble, and the remaining 30% is pectin **[5, 6]**. Among vitamins, they contain primarily vitamin C (4.6-5 mg/100 g), as well as potassium (107-112 mg/100 g) **[5]**.

Although the antioxidant capacity of apples is less than that of berries, it is still relatively high, and in terms of phytonutrients they have an outstanding polyphenolic compound content **[7]**. Polyphenolic compounds mostly accumulate in the flesh, under the skin **[8]**, and their amount may reach values of 2 to 3,000 mg/kg. The dominant compounds are catechins, epicatechins, cyanidins and quercetin **[9]**.

Polyphenols

Phenolic compounds are secondary metabolites of plants. They are responsible for the chemical protection of the individual plants against pests, and for the interference between plant and plant **[10]**. They occur widely in the flora and can be found in many edible plants. More than 8,000 compounds have already been identified **[11]**. Fruit polyphenols include flavonoids, phenolic acids and tannins, among other things **[12]**. In foods, they are often responsible for the development of color, sour and bitter smell or taste **[13]**. During food production, they can cause discoloration and turbidity as they form complexes with metals and proteins **[14]**.

These compounds are primary (chain-breaking) antioxidants **[15]**, they can help prevent cardiovascular and neurodegenerative diseases, and may have a preventive effect on certain cancers **[16, 17, 18]**. Due to their antimicrobial and antioxidant effects, they can help to increase shelf life in the food industry **[19]**.

Free radicals and antioxidants

Free radicals are atoms or molecules whose outer electron shell contains one or more unpaired electrons, or their energy levels are higher than normal and therefore they are highly reactive. Consequently, they can trigger redox reactions that damage especially lipids, proteins and nucleic acids that build up living organisms **[20]**. They are usually molecules or molecule parts with oxygen, nitrogen, sulfur or carbon atoms at their center **[21]**. Depending on the conditions or their quantity, they may act as oxidizing or reducing agents, they can transfer their unpaired electron to another atom or molecule, but they can also pick one up from the reaction partner **[22]**.

An antioxidant is any substance that is present in small amounts compared to the oxidizable substrate, but can significantly reduce or even inhibit the oxidation of the substrate **[23]**. The task of the antioxidant protection system is to protect living organisms from damage caused by free radicals in such a way as to minimize the amount of free radicals that form in the body or counteract their harmful effects. This system is organized on several levels: it comprises antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic compounds (e.g., antioxidant vitamins: vitamins C, E and A, polyphenolic compounds, carotenoids, etc.) **[24]**.

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However, it is important to note that, based on recent studies, there has been a paradigm shift regarding the significance and applicability of antioxidant capacity results obtained using *in vitro* methods, because these methods do not provide sufficient information on the actual bioavailability of the compounds. Due to the limitations of the methods, such as the nature of the free radicals examined, the results of „test tube experiments" cannot be directly applied to *in vivo* health effects. At the same time, *in vitro* antioxidant capacity is an important indicator of the quality of certain foods and has a direct effect on the inhibition of oxidation processes in foods **[25, 26]**.

Patulin

Chemically, patulin is an unsaturated lactone produced by certain species (e.g., *Aspergillus clavatus, Penicillium expansum* and *Byssochlamys nivea)* of *Penicillum*, *Aspergillus* and *Byssochlamys* strains. It has been proven to damage membranes, after breaking up the plasma membrane it reacts with the sulfhydryl group of proteins and amino acids, thereby altering the functioning of enzymes. Acute toxicity is rare, but it can cause a number of different symptoms, including restlessness, seizures, vascular congestion, edema, digestive and respiratory symptoms. In severe cases, internal bleeding or cerebral edema may develop. In chronic cases (in the case of long-term exposure) it is genotoxic, immunotoxic, may damage the kidneys and disturb protein synthesis **[27]**. According to the classification of the *International Agency for Research on Cancer*, it belongs to Group 3, i.e., it is not classifiable as to its carcinogenicity to humans, because there is not enough information on the carcinogenic effects of the compound **[28]**.

The amount of patulin in foods is legally regulated. According to Commission Regulation (EC) No 1881/2006 the maximum level of patulin in fruit juices and spirit drinks is 50 µg/kg, in solid apple products it is 25 µg/kg, and in baby foods it is 10 µg/kg. The maximum tolerable daily intake is 0.4 µg/kg bw **[29]**.

Hydroxymethylfurfural

5-(Hydroxymethyl)furfural (HMF) is produced in carbohydrate-containing foods in the Maillard reaction that takes place between simple sugars (glucose, fructose) and amino groups, especially at low pH values and high temperatures. It is not present in fresh foods and its formation can be linked to heat treatment, so it can be an indicator compound of the extent of heat treatment the food has been subjected to or of storage conditions **[30]**. In animal studies, its cute toxicity is low, its carcinogenicity is not unequivocally proven based on the evidence **[31]**. In the body, it can be converted into sulfoxymethylfurfural (SMF), and so it may be indirectly genotoxic **[32]**.

Materials and methods

Test samples

For the tests, 12 different 100% apple juices, commercially available in Hungary, were used, five of which were made from concentrate, while seven of them were plain press juices. Three of the press juice products came from organic farms. Apple juices were purchased from retail stores two weeks prior to the start of the experiments. 4 to 10 liters of each product were purchased in 1 to 5 liter sizes, making sure that the samples of each product come from the same batch, so that they have the same date of minimum durability. Characteristics of the samples are shown in **Table 1**.

Chemicals and instruments

Spectrophotometric methods were performed using a Thermo Helios Alpha UV-VIS (±0.001 absorbance unit) spectrophotometer with cuvettes of 1 cm path length.

The methanol and 96% ethanol used for the tests were purchased from Sigma-Aldrich. Hydroxymethylfurfural (Acros), ascorbic acid (VWR), gallic acid (Sigma-Aldrich) and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich) were used as standards.

In addition, barbituric acid (Acros), p-toluidine (Sigma-Aldrich), propanol (Sigma-Aldrich), Folin-Ciocalteu reagent (Sigma-Aldrich), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine, Sigma-Aldrich), iron chloride hexahydrate (Sigma-Aldrich), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, Sigma-Aldrich), potassium persulfate (Acros Organics), copper(II) chloride (Alfa Aesar), neocuproine (2,9-dimethyl-1,10-phenanthroline, Sigma-Aldrich), sodium carbonate (Sigma-Aldrich), sodium acetate (Sigma-Aldrich), acetic acid (Sigma-Aldrich), ammonium acetate (Molar Chemicals Ltd.), hydrochloric acid (Carlo Erba) and distilled water were used for the reactions.

Analytical methods

Patulin contamination

Patulin contamination was analyzed by an HPLC-UV method in the Food Toxicology National Reference Laboratory of Nébih, following acetonitrile extraction and a special clean-up. The method is accredited by the National Accreditation Authority for plant matrices, with the ID no. PAT/HPLC/87/2013 **[33]**. Results are given in μg/kg.

Total dry matter content, total soluble dry matter content

Total dry matter content was determined by gravimetry after drying at 60 °C in a drying oven, total soluble dry matter content was determined by refractometry, using a Zeiss-Abbe refractometer. Results are given in percent.

Ash content

Ash content was determined by a gravimetric method, after incineration on an open flame in a furnace, following ignition at 600 °C. Results are given in percent.

Hydroxymethylfurfural content

Hydroxymethylfurfural content was determined by a spectrophotometric method based on the color reaction of the compound with barbituric acid and p-toluidine (Winkler method) **[34]**. Results are given in mg/l.

Antioxidant capacity

Sample preparation: In the case of unfiltered apple juice samples, the analysis was carried out after centrifugation from the supernatant. I order to perform the measurement in the appropriate calibration range, dilution tests were carried out, and on the basis of these, measurement of the antioxidant activity and polyphenolic compound content of apple juices was carried out after dilution of the samples with distilled water.

FRAP method: The principle of the method is that the iron-2,4,6-tripyridyl-s-triazine (Fe^{3+}-TPTZ) complex is reduced by antioxidants at low pH to the Fe^{2+} complex **[35]**. This reaction is accompanied by a color change, during which the original yellowish brown complex turns blue, with an absorption maximum of 595 nm **[36]**. Results are given in ascorbic acid equivalent *[AaEq]*, in mg/l.

CUPRAC method: Due to the reducing ability of antioxidants, the oxidation number of the Cu^{2+} ion of dissolved $CuCl_2$ decreases. The resulting Cu^{1+} dimerizes the neocuproine in the reaction mixture at pH 7, thus turning it into a yellowish compound. The color change can be followed by a spectrophotometer at a wavelength of 450 nm **[37]**. Results are given in trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent *[TEq],* in μmol/l.

DPPH method: Antioxidants react with the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical in the reaction mixture, as a result of which the compound loses its original dark purple color, and this change can be followed at 517 nm. The more antioxidant type compounds are found in a given volume sample, the more intense the color loss is **[38]**. Results are given in trolox equivalent *[TEq]*, in μmol/l.

ABTS method: The method is based on the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The potassium persulfate used for the reaction forms a colored free radical from ABTS at pH 7.4, which is captured by the antioxidants of the sample. This color change can be followed at 734 nm **[39]**. Results are given in trolox equivalent *[TEq],* in μmol/l.

Total phenolic content (TPC)

The Folin-Ciocalteau method was used for the analysis. The full chemical background of the reaction is unknown. The Mo(VI) in the Folin reagent reacts with the reducing compounds, such as polyphenolic compounds, of the test sample at pH 10. During the reaction, blue Mo(V) is formed, with an absorption maximum at 750 nm **[40]**. Results are given in gallic acid equivalent *[GaEq]*, in mg/l.

Data analysis

During data analysis, the Mann-Whitney test and one-way analysis of variance were used with a Tukey post hoc test to reveal the differences between the samples **[41]**, using the XLStat software (Addinsoft, Paris, France).

The principle of the sum of ranking differences (SRD) method was described by Héberger, while its validation and software implementation were completed by Héberger and Kollár-Hunek **[42, 43]**. The non-parametric method does not require the condition of normality in the data set. One of the milestones in the development of the SRD method was the possibility of analyzing data with binding, which is described in detail in the 2013 publication of Kollár-Hunek and Héberger **[43]**. The macro written in Microsoft Excel VBA environment requires, as an input format, the ordering of cases (in our case, the results of the measurement methods) in rows and the variables to be compared (apple juice products) in columns. There are four options for comparison, which are handled by the software as reference values: average (Ave), maximum (Max), minimum (Min) or read value (Read). In our work, the read value was used, so for each measurement result the best one was selected as reference. The SRD method is based on the fact that the closer the ranking of the results of a particular sample is to the reference/standard SRD value, the more it resembles the given reference. The SRD method is freely downloadable via the following link: http://aki.ttk.mta.hu/srd/ .

Results

Patulin content

Detectable contamination was found in one sample only: the amount of mycotoxin in the product was 28 μg/kg. In the case of the other samples, the patulin content was below the LOQ *(Limit of Quantitation* **[44]***)* of 6.8 μg/kg of the method used.

Total dry matter content and soluble dry matter content

The total dry matter content was in the range of 12.11 to 15.04%, and it was generally higher in the case of press juices. The maximum of the total soluble dry matter content was around 10% for each product. There was a significant difference between the product groups in the case of both parameters. The results are shown in **Figure 1**.

Ash content

By determining the ash content, information is obtained about the inorganic content of the products, which are made up of the natural constituents of apple juices (macroelements, trace elements) and possible impurities.

As can be seen in **Figure 2**, the ash content of the apple juices examined varies from 0.13 to 0.24%. The highest and lowest values were found in the case of products prepared from concentrates, while the difference between the values of the two product groups was not significant.

Hydroxymethylfurfural content

The hydroxymethylfurfural (HMF) content is an indicator of the extent of heat treatment the product has been subjected to: the higher the HMF content, the more intense the heat treatment of the given apple juice has been. The amount of the compound was greatest in a press juice (4.51 mg/l), followed by two apple juices made from concentrate (3.25 and 2.57 mg/l). It is present in the lowest amount in a product manufactured by pressing (1.50 mg/l). In the case of the other press juices, the HMF content is about the same, the amount being lower than that of products prepared from concentrate. For the latter product group, there are greater differences between the products in terms of the amount of the compound. There were significant differences between the products. The HMF content of the apple juices is shown in **Figure 3**.

Antioxidant capacity

In the case of antioxidant capacity, different tendencies could be observed for the two groups: the values for press juices were higher. The same product of the product group exhibited the highest value in the case of all of the methods (1626.20 mg/l [AaEq]; 9323.29 µmol/l [TEq]; 5188.02 µmol/l [TEq]; 6839.82 µmol/l [TEq]). Among the products prepared from concentrate, the values of the product with the highest antioxidant capacity were one fifth to one quarter of the above (388.30 mg/l [AaEq]; 3542.25 µmol/l [TEq]; 965.03 µmol/l [TEq]; 1394.99 µmol/l [TEq]).

The antioxidant capacity of a product prepared from concentrate was the lowest both in the product group and among all of the samples tested (85.07 mg/l [AaEq]; 527.29 µmol/l [TEq]; 287.13 µmol/l [TEq]; 327.79 µmol/l [TEq]). The values of the product with the highest antioxidant capacity were approximately twenty times higher. The results obtained are shown in **Table 2**.

Total phenolic content

In terms of polyphenol content, the two product groups are clearly separated: the values for press juices are several times higher than those of products prepared from concentrate. Products within the same group also differ from each other significantly: the value for the product with the highest polyphenol content (2351.08 mg/l [GaEq]) is approximately three times higher than that of the product with the lowest value within the group (819.54 mg/l [GaEq]).

There is a similarly large difference between the products prepared from concentrate. The polyphenol content of the product with the highest value (770.48 mg/l [GaEq]) is more than four times higher than that of the product with the lowest polyphenol content (186.32 mg/l [GaEq]). Similarly to the antioxidant capacity values, this product has the lowest polyphenol content of all the products tested. The product with the highest value possesses levels that are approximately twelve times higher. Total phenolic compound results are shown in **Figure 4**.

Results of statistical analysis

Based on teh correlation analysis, there is a strong relationship between the results of antioxidant capacity measurements and the total phenolic content. Results of the Spearman rank correlation are shown in **Table 3**, in the correlation matrix.

With the help of the SRD method, the best result was selected for all the parameters measured. These are the highest total dry matter content, in the case of the total water-soluble dry matter content the Brix value closest to the Brix value of 11.2% for fruit juices prepared from apples, as given in guideline 1-3-2001/112 of the Hungarian Food Codex, the highest ash content, the lowest hydroxymethylfurfural content, the highest antioxidant capacity value for each method, and the highest total phenolic content value. With their use, an imaginary reference product was created by the method, to which the products tested are compared. Results are shown in **Figure 5**.

Three press juices (P1, P2, P5) are most similar to the reference product, these are followed closely by the other four products of the product group (P3, P4, P6, P7). Of the products prepared from concentrate, one is outstanding (K1), while the other four products of the group from two groups (K4, K5 and K2, K3).

It can be seen that the product groups are clearly separated by the method, however, the products within the groups also differ from each other, which is probably due to the differences between the fruits used as raw materials and their qualities. Based on the parameters investigated, product K1 is located at the border of the two categories.

On the basis of the analytical parameters investigated and the statistical analysis, it can be stated that press juices are more beneficial than products prepared from concentrate from a nutrition physiology point of view.

Discussion

In the course of this research, apple juices with a fruit content of 100%, produced using different technologies, were investigated. The parameters studied in the laboratory were patulin contamination, dry matter and ash content, hydroxymethylfurfural content, antioxidant capacity and polyphenolic compound content.

In terms of patulin contamination, the situation is different in countries around the world. According to a study from 2000 to 2011, processing Spanish market product data, mycotoxin contamination of apple juice products from non-EU countries exceeded the EU limit value **[45]**. An Iranian study showed that in 37.5% of 8 apple juice products available in supermarkets the patulin content was higher than 50 μg/kg **[46]**. In Tunisia, 27 of 42 apple juices were contaminated **[47]**.

Products from EU member states usually comply with regulations. In a study conducted in Portugal, the amount of patulin in apple juices purchased in local supermarkets and organic stores was below the limit value, did not reach the LOQ value or was below the limit of detection **[48]**. The patulin content of apple juices produced in or imported by Belgium did not exceed the limit value either, and no statistically significant difference was found between the contamination of press juices and traditional apple juices **[49]**.

As a counterexample, Romania can be mentioned, where 6% of 50 apple-based products had mycotoxin levels higher than the upper limit value **[50]**.

In a Hungarian study, the contamination of 24 apple juice concentrates was checked. Patulin content was below the upper limit value for all of the samples **[51]**.

The apple juice samples examined by us came from Hungary and from EU member states. Patulin contamination exceeded the LOQ (Limit of Quantitation) value in the case of only one of the 12 samples with the method used by the NFCSO. In this one sample the amount of the patulin was 28 μg/kg, which is below the European Union limit of 50 μg/kg. The fact that the extent of contamination is lower in EU member states is probably due to stricter regulations for the agricultural sector and the food industry.

It is noteworthy that although, according to the literature, the amount of mycotoxin in most products does not reach 50 μg/kg, in many cases it exceeds the upper limit value of 10 μg/kg in products intended for children and the maximum daily intake value. This can raise food safety issues if these products are regularly consumed by children. At the same time, based on our own measurement results, mycotoxin contamination only occurs in very rare cases in the domestic commercial sector.

Hydroxymethylfurfural (HMF) can be used as an indicator of the extent of heat treatment of fruit juices **[52]**. According to literature data, the compound is present in apple juices in varying amounts.

In a storage experiment, the initial HMF content of the three apple juice samples tested were 4.39 mg/l, 1.86 mg/l and 28.55 mg/l, respectively, which increased significantly during storage at elevated temperatures **[53]**.

According to the results of a comparative study of apple juices made from concentrate and so-called *cloudy apple juice* (unfiltered apple juice) products, the HMF content was 0.57-3.5 mg/l for the former product group, while it was between 0.36 and 0.48 mg/l for the latter group **[54]**.

In a Canadian study, apple juices made from concentrate and from fresh fruit were used: in fresh products, the amount of the compound was between 0 and 2.55 mg/l, while in the products made from concentrate it was between 0.4 and 2.3 mg/l **[55]**.

The HMF content of the apple juices used in our research showed a variation similar to literature data, however, the trend observed (higher HMF content detected in products prepared from concentrate) is the same as in the literature. The differences can mainly be due to the different production technologies of the product groups and the products and to different storage conditions.

In terms of the *in vitro* antioxidant capacity, literature data show great differences. Since there is no universal laboratory method for the analysis of this parameter, instead of the exact values, it is better to observe the differences between the average values for the products and product categories.

Antioxidant activity is significantly influenced by the apple variety used as the raw material. The antioxidant activity of the Red Delicious variety, often used for the production of apple juices, is higher than that of the also popular Golden Delicious or Idared varieties **[56]**.

Activity values show a clear decrease during production. According to a study, the antioxidant activity of freshly pressed apple juice is 3-10% of that of the whole fruit, in the case of several apple varieties **[57]**.

Based on a study comparing 90 products, the antioxidant capacity of unfiltered apple juices prepared from concentrate and not from concentrate, as measured by the TEAC (Trolox Equivalent Antioxidant Capacity) method, was much higher on average (2.8-7.1 and 2.1-17 mmol/l [TEq]) than of filtered products prepared from concentrate (1.1-4.6 mmol/l [TEq]). Our own measurements produced similar values **[58]**.

Significant differences were observed in this parameter in the case of filtered and unfiltered apple juices that underwent enzymatic and microfiltration pectin removal procedures. Microfiltration was more favorable for preserving antioxidant activity, however, the values for unfiltered juices were higher both in the case of the enzymatic and the filtration procedure **[59]**.

The antioxidant capacity of the apple juices examined by us showed a tendency similar to that of the literature: products prepared by pressing achieved higher values in the case of all of the methods used.

Polyphenolic compounds possess antioxidant properties as well, so the total phenolic content is closely related to the antioxidant capacity **[11]**. Compared to the entire range of fruits, the polyphenolic compound content of apples is not outstanding, but it can reach even 2,000-3,000 mg/kg [GaEq] **[9]**. The variety of the apple is also an important factor from this point of view as well **[56]**.

In the case of apple juices prepared in the laboratory, the total polyphenolic compound content of the unfiltered type was nearly one and a half times higher than that of filtered products: in the case of the Champion apple variety, the value was 1044.4 mg/l for the former and 689.5 mg/l for the latter **[60]**.

One study compared the total phenolic content of 24 commercially available apple juices. In the case of filtered apple juices, the compounds were present in amounts of 109.9, 165.5 and 172.7 mg/l [GaEq]. In unfiltered products, the value varied widely, ranging from 152.2 to 459.0 mg/l [GaEq] **[61]**.

The 90-product research already mentioned in the antioxidant activity section showed a similar tendency for polyphenolic compounds: the polyphenol content of unfiltered apple juices prepared from concentrate and not from concentrate was higher (385-757 and 171-1925 mg/l [GaEq]) than that of their filtered counterparts prepared from concentrate (116-448 mg/l [GaEq]) **[58]**.

In the case of the apple juices examined in the course of this research, the total polyphenolic compound content results were similar to those described in the literature: pressed juices had a higher polyphenol content.

Based on the statistical analysis of our results, the antioxidant capacity and the total phenolic content are closely related, i.e., the antioxidant properties of apples are mainly determined by their polyphenols. This is also supported by the results of other studies **[58, 62, 63]**.

Conclusions

Based on the results of our research work and literature data, the following conclusions can be drawn.

Based on the results of patulin contamination analyses, products on the domestic market comply with Commission Regulation (EC) No 1881/2006, the overall picture is reassuring in terms of food safety.

During the analysis of the dry matter content, significant differences were found between the two product groups. Press juices have a higher total dry matter content, which is obviously due to the fiber content.

The ash content of the different products varies within a relatively wide range, however, there is no significant difference between the product categories based on the test results. This may indicate that the parameter is less influenced by technological differences than by the variety and quality of the apples used as raw material.

The hydroxymethylfurfural (HMF) content is highly dependent on the manufacturing technology, particularly on the extent of heat treatment the product is subjected to, as confirmed by our own research results. The amount of the compound was generally higher in apple juices prepared from concentrate, however, an outstanding value was observed in a press juice. According to information obtained from the manufacturer, the product is subjected to so-called „prolonged heat treatment", that is to say, after the heat treatment, the apple juice is filled into the packaging material without cooling, and it is then allowed to cool slowly at the temperature of the plant. This procedure is also used in the case of another press juice examined by us, but the value was lower in that case.

The amount of HMF in the product is also influenced by the storage conditions. During prolonged storage at elevated temperatures, the amount of the compound has increased significantly in the apple juices **[53]**. It is likely that the higher HMF content of the product analyzed by us is due to the different storage conditions.

The close correlation between the antioxidant capacity and the total phenolic content indicates the dominance of polyphenolic compounds in the development of the antioxidant capacity of apple juices, which is also supported by literature data **[58, 62, 63]**.

Press juices have a higher polyphenolic compound content, which is probably due to the more gentle production technology. This is indicated by the lower hydroxymethylfurfural content.

As a final conclusion, it can be stated that, based on the laboratory parameters examined by us, the statistical analyses and literature data, the consumption of 100% fruit content apple juices can have a beneficial effect on our health. The products are safe, their consumption is beneficial due to the preserved health-protecting components. From a nutrition physiology point of view, press juices are a better choice because of their fiber content and outstanding polyphenolic compound content, which is due to the more gentle production technology.

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References

[1] Tóth M. (2014): *Magyarország kultúrflórája - Az alma.* Agroinform Kiadó és Nyomda Kft., Budapest.

[2] Bubán T., Glits M., Gonda I., G. Tóth M., Harmat L., Hrotkó K., és mtsai. (2004): *A gyümölcsök termesztése.* Mezőgazda Kiadó, Budapest.

[3] Központi Statisztikai Hivatal (2017. október 20): *4.1.18. A fontosabb gyümölcsfélék termesztése és felhasználása (2014–).* http://www.ksh.hu/docs/hun/xstadat/xstadat_eves/i_omn006a.html Hozzáférés/Acquired: 02. 12. 2017.

[4] Roszík P. (2017): *Jelentés a Biokontroll Hungária Nonprofit Kft. 2016. évi tevékenységéről.* Biokontroll Hungária Nonprofit Kft., Budapest.

[5] United States Department of Agriculture, Agricultural Research Service, USDA Food Composition Databases: *National Nutrient Database for Standard Reference Release 28.* https://ndb.nal.usda.gov/ndb/search Hozzáférés/Acquired: 02. 12. 2015.

[6] Salunkhe D. K., Kadam S. S. (1995): *Handbook of Fruit Science and Technology.* Marcel Dekker Inc., New York.

[7] Balázs A., Tóth M., Blazics B., Héthelyi É., Szarka S., Ficsor E., és mtsai. (2012): Investigation of dietary important components in selected red fleshed apples by GC–MS and LC–MS. *Fitoterapia* **83** (8) 1356-1363.

[8] Bhagwat S., Haytowitz D. B., Holden J. M. (2013. december): *USDA Database for the Flavonoid Content of Selected Foods Release 3.1.* United States Department of Agriculture: https://www.ars.usda.gov/ARSUserFiles/80400525/Data/Flav/Flav_R03-1.pdf Hozzáférés/Acquired: 28. 12. 2017.

[9] Kalinowksa M., Bielawska A., Lewandowska-Siwkiewicz H., Priebe W., Lewandowski W. (2014): Apples: Content of phenolic compounds vs. variety, part of apple and cultivation model, extraction of phenolic compounds, biological properties. *Plant Physiol. Bioch.* **84** 169-188.

[10] Gutiérrez-Grijalva E. P., Ambriz-Pére D. L., Leyva-López N., Castillo-López R. I., & Heredia J. B. (2016): Review: dietary phenolic compounds, health benefits and bioaccessibility. *Arch. Latinoam. Nutr.* **66** (2)

[11] Yang C. S., Sang S., Lambert J. D., Lee M. J. (2008): Bioavailability issues in studying the health effects of plant polyphenolic compounds. *Mol. Nutr. Food Res.* **52** 139 –151.

[12] Haminiuk C. W., Maciel G. S., Plata-Oviedo M. S., Peralta R. M. (2012): Phenolic compounds in fruits – an overview. *Int. J. Food Sci. Tech.* **47** 2023-2044.

[13] Shahidi F., Naczk N. (2004): *Phenolics in Food and Nutraceuticals.* Boca Raton: Taylor & Francis Group, LLC.

[14] Belitz H.-D., Grosch W., Schieberle P. (2009): *Food Chemistry.* Berlin Heidelberg: Springer-Verlag GmbH.

[15] Alamed J., Chaiyasit W., McClements D. J., Decker E. A. (2009): Relationships between free radical scavenging and antioxidant activity in foods. *J. Agr. Food Chem.* **57** (7) 2969-2976.

SCIENCE

[16] Castañer O., Covas M. I., Khymenets O., Nyyssonen K., Konstantinidou V., Zunft H. F., és mtsai. (2012): Protection of LDL from oxidation by olive oil polyphenols is associated with a downregulation of CD40-ligand expression and its downstream products in vivo in humans. *Am. J. Clin. Nut.* **95** (5) 1238–1244.

[17] Bastianetto S., Ménard C., & Quirion R. (2015): Neuroprotective action of resveratrol. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1852** (6) 1195-1201.

[18] Roleira F. M., Tavares-da-Silva E. J., Varela C. L., Costa S. C., Silva T., Garrido J., és mtsai. (2015): Plant derived and dietary phenolic antioxidants: Anticancer properties. *Food Chem.* **183** 235-258.

[19] Daglia M. (2012): Polyphenols as antimicrobial agents. *Curr. Opin. Biotech.* **23** (2) 174-181.

[20] Pacifici R. E., Davies K. J. (1991): Protein, Lipid and DNA Repair Systems in Oxidative Stress: The Free-Radical Theory of Aging Revisited. *Gerontology* **37** 166–180.

[21] Cadenas E. (1989): Biochemistry of Oxigen Toxicity. *Annu. Rev. Biochem.* **58** 79-110.

[22] Cheeseman K. H., Slater T. F. (1993): An introduction to free radical biochemistry. *Brit. Med. Bull.* **49** (3) 481-493.

[23] Halliwell B., Gutteridge J. M. (2015): *Free Radicals in Biology and Medicine.* Oxford University Press, Oxford.

[24] Benzie I. F. (2000): Evolution of antioxidant defence mechanisms. *Eur. J. Nutr.* **39** (2) 53-61.

[25] Pompella A., Sies H., Wacker R., Brouns F., Grune T., Biesalski H. K., és mtsai. (2014): The use of total antioxidant capacity as surrogate marker for food quality and its effect on health is to be discouraged. *Nutrition* **30** (7-8) 791-793.

[26] Fraga C. G., Oteiza P. I., Galleano M. (2014): *In vitro* measurements and interpretation of total antioxidant capacity. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1840** (2) 931-934.

[27] Babaali E., Abbasi A., Sarlak Z. (2017): Risks of Patulin and its Removal Procedures: Review. *International Journal of Nutrition Sciences* **1** 9-14.

[28] IARC (1987): *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans - List of Classification,Volume 40, Sup 7.* International Agency for Research on Cancer: http://monographs.iarc.fr/ENG/Classification/latest_classif.php Hozzáférés/Acquired: 04. 12. 2017.

[29] A Bizottság 1881/2006/EK rendelete (2006. december 19.) az élelmiszerekben előforduló egyes szennyező anyagok felső határértékeinek meghatározásáról. http://eur-lex.europa.eu/legal-content/HU/TXT/?uri=celex:32006R1881 Hozzáférés/Acquired: 02. 12. 2017.

[30] Stadler R. H., Lineback D. R. (2008): *Process-Induced Food Toxicants: Occurrence, Formation, Mitigation, and Health Risks.* John Wiley & Sons Inc., Hoboken, New Jersey.

[31] Abraham K., Gürtler R., Berg K., Heinemeyer G., Lampen A., Appel K. E. (2011): Toxicology and risk assessment of 5-Hydroxymethylfurfural in food. *Mol. Nutr. Food Res.* **55** (5) 667-678.

[32] Capuano E., Fogliano V. (2011): Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT–*Food Sci. Technol.* **44** (4) 793-810.

[33] Nemzeti Akkreditáló Hatóság (2015. június 15): MÓDOSÍTOTT RÉSZLETEZŐ OKIRAT (2) a NAH-1-1160/2015 nyilvántartási számú akkreditált státuszhoz. NÉBIH ÉTbI Élelmiszer Toxikológiai Nemzeti Referencia Laboratórium (ÉTNRL): http://airterkep.nebih.gov.hu/gis_portal/laborok/okiratok/etbi/Toxikologia_NAH-1-1160_2015_MRO2.pdf Hozzáférés/Acquired: 08. 02. 2018.

[34] Winkler O. (1955): Beitrag zum Nachweis und zur Bestimmung von Oxymethylfurfurol in Honig und kunsthonig. *Z. Lebensm. Unters. For.* **102** (3) 161-167.

[35] Huang D. J., Ou B. X., Prior R. L. (2005): The chemistry behind antioxidant capacity assays. *J. Agr. Food Chem.* **53** (6) 1841-1856.

[36] Benzie I. F., Strain J. J. (1996): The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem* **239** 70-76.

[37] Apak R., Güçlü K., Özyürek M., Çelik S. E. (2008): Mechanism of antioxidant capacity assays and the CUPRAC (Cupric ion Reducing Antioxidant Capacity) assay. *Microchim. Acta* **160** (4) 413-419.

[38] Brand-Williams W., Cuvelier M., Berset C. (1995): Use of a free radical method to evaluate antioxidant activity. *LWT – Food Sci. Technol.* **28** (1) 25-30.

[39] Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio. Med.* **26** (9-10) 1231-1237.

[40] Singleton V. L., Rossi Jr. J. A. (1965): Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Viticult.* **16** (3) 144-158.

[41] Sajtos L., Mitev A. (2007): *SPSS Kutatási és adatelemzési kézikönyv.* Alinea Kiadó, Budapest.

[42] Héberger K. (2010): Sum of ranking differences compares methods or models fairly. *TRAC - Trend. Anal. Chem.* **29** (1) 101-109.

[43] Kollár-Hunek K., Héberger K. (2013): Method and model comparison by sum of ranking differences in cases of repeated observations (ties). *Chemometr. Intell. Lab.* **127** 139-146.

[44] Pokol G., Gyurcsányi R. E., Simon A., Bezúr L., Horvai G., Horváth V., és mtsai. (2017): *Analitikai kémia.* Typotex, Budapest.

[45] Marín S., Mateo E. M., Sanchis V., Valle-Algarra F. M., Ramos A. J., Jiménez M. (2011): Patulin contamination in fruit derivatives, including baby food, from the Spanish market. *Food Chem.* **124** 563-568.

[46] Azizi I. G., Rouhi S. (2013): Determination of patulin in fruit juices and compote of apple and pear. *Toxin Rev.* **32** (3) 39-42.

[47] Zouaoui N., Sbaii N., Bacha H., Abid-Essefi S. (2015): Occurrence of patulin in various fruit juice marketed in Tunisia. *Food Control* **51** 356-360.

[48] Cunha S. C., Faria M. A., Pereira V. L., Oliveira T. M., Lima A. C., Pinto E. (2014): Patulin assessment and fungi identification in organic and conventional fruits and derived products. *Food Control* **44** 185-190.

[49] Tangni E. K., Theys R., Mignolet E., Maudoux M., Michelet J. Y., Larondelle Y. (2003): Patulin in domestic and imported apple-based drinks in Belgium: occurrence and exposure assessment. *Food Addit. Contam.* **20** (5) 482-489.

[50] Oroian M., Amariei S., Gutt G. (2014): Patulin in apple juices from the Romanian market. *Food Addit. Contam.* B **7** (2) 147-150.

[51] Boross F., Tóth T. (2007): A magyar almalé-sűrítmények vizsgálata. Élelmiszervizsgálati *Közlemények* **53** (2) 88-94.

[52] Gökmen V., Acar J. (1999): Simultaneous determination of 5-hydroxymethylfurfural and patulin in apple juice by reversed-phase liquid chromatography. *J. Chromatogr. A* **847** (1-2) 69-74.

[53] Karatas S., Akkaya D. (2017): Effect of storage temperature on Hydroxymethylfurfural contents in apple juice. *Chemistry Research Journal* **2** (1) 12-19.

[54] Marsol-Vall A., Balcells M., Eras, J., Canela-Garayoa R. (2016): A rapid gas chromatographic injection-port derivatization method for the tandem mass spectrometric determination of patulin and 5-hydroxymethylfurfural in fruit juices. *J. Chromatogr. A* **1453** 99-104.

[55] Kermasha S., Goetghebeur M., Dumont J., Couture R. (1995): Analyses of phenolic and furfural compounds in concentrated and non-concentrated apple juices. *Food Res. Int.* **28** (3) 245-252.

[56] Boyer J., Liu R. H. (2004): Apple phytochemicals and their health benefits. *Nutr. J.* **3** (5)

[57] van der Sluis A. A., Dekker M., Skrede G., Jongen W. M. (2002): Activity and Concentration of Polyphenolic Antioxidants in Apple Juice. *J. Agr. Food Chem.* **50** (25) 7211–7219.

[58] Włodarska K., Pawlak-Lemańska K., Górecki T., Sikorska E. (2017): Classification of commercial apple juices based on multivariate analysis of their chemical profiles. *Int. J. Food Prop.* **20** (8) 1773-1785.

[59] Markowski J., Baron A., Le Quéré J.-M., Płocharski W. (2015): Composition of clear and cloudy juices from French and Polish apples in relation to processing technology. *LWT – Food Sci. Technol.* **62** (1) 813-820.

[60] Oszmianski J., Wolniak M., Wojdylo A., & Wawer I. (2007): Comparative study of polyphenolic content and antiradical activity of cloudy and clear apple juices. *J. Sci. Food Agr.* **87** 573-579.

[61] Kahle K., Kraus M., Richling E. (2005): Polyphenol profiles of apple juices. *Mol. Nutr. Food Res.* **49** 797-806.

[62] Schempp H., Christof S., Mayr U., Treutter D. (2016): Phenolic compounds in juices of apple cultivars and their relation to antioxidant activity. *J. Appl. Bot. Food Qual.* **89** 11-20.

[63] Gliszczynska-Swiglo A., Tyrakowska B. (2003): Quality of Commercial Apple Juices Evaluated on the Basis of the Polyphenol Content and the TEAC Antioxidant Activity. *J. Food Sci.* **68** (5) 1844-1849.