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POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY OF FRUIT AND VEGETABLE BEVERAGES PROCESSED BY DIFFERENT TECHNOLOGY METHODS

Jiří Mlček, Tünde Juríková, Soňa Škrovánková, Marcela Paličková, Jana Orsavová, Ladislava Mišurcová, Irena Hlaváčová, Jiří Sochor, Daniela Sumczynski

ABSTRACT

The purpose of the natural drinks production is the preservation of biologically active compounds in maximal amount in prepared drinks. The issue is the loss of these substances due to conventional conservation methods, such as pasteurization. Pascalization, a conservation method using high pressure, performs a new trend in conservation. According to available research, it causes only a minimal loss of bioactive compounds. Influence of conservation technology of fruit and vegetable beverages on the content of bioactive substances – polyphenols, flavonoids and on their antioxidative activity has been investigated. Their content has been compared in fresh juice samples, in samples conserved by pasteurization and after the appliance of high pressure treatment – pascalization (HPP). HPP has a positive effect on total antioxidative capacity of juices – broccoli with apple (increase of the amount from 189.12 mg.100 mL^{-1} to 217.12 mg.100 mL^{-1}) and beetroot and on total polyphenol content within all samples of beverages except from carrot juice. Decrease of the amounts of flavonoids has been observed within all beverages. For drinks after pasteurization treatment there is evident the decrease of total polyphenols content and total antioxidant activity, besides carrot juice, where the antioxidant capacity value had increased from 37.24 to 43.14 mg.100 mL^{-1}. The flavonoid content of fruit and vegetable juices after heat treatment had increased only in the juice prepared from broccoli with apple (from 40.71 mg.100 mL^{-1} to 45.14 mg.100 mL^{-1}), the content in other juices had decreased. However, the decrease of the flavonoid content is lower after heat treatment in comparison to HPP, except the samples of cabbage juice with apple. With the exception of flavonoids, HPP has been proved as a gentle conservation technology enabling preserving higher amounts of bioactive substances with antioxidative properties if compared with the heat treatment. For the samples treated by HPP there was observed statistically significant difference in comparison with fresh juice in all factors mentioned above (p <0.05).

Keywords: beverages; pasteurization; pascalization; polyphenols; antioxidative activity

INTRODUCTION

Fruit and vegetable contain a significant amount of biologically active substances able to lower a risk of any type of cancer or other civilization diseases **(Mendelová et al., 2016; Jakubcova et al., 2014)**. These bioactive substances are engaged in biochemical processes and are significant parts of the immune system **(Turati et al., 2015; Paliyath, 2011)**.

One of the postulate held by supporters of natural beverages is the preservation of the maximal amount of biologically active substances in processed juices. The issue is the loss of these substances due to conventional conservation methods, such as pasteurization. Pascalization, a conservation method using high pressure, performs a new trend in conservation. According to available research, it causes only a minimal loss of volatile compounds, pigments, vitamins, antioxidant compounds, and mineral substances **(Norton and Sun, 2008; Ramirez et al., 2009)**.

Conservation by high pressure (100 – 1000 MPa), high pressure processing (HPP), is a relatively new conservative method enabling preserving food natural properties and content of nutritional substances with health benefits using non-thermal processing to paralyze harmful pathogens and spoilage microorganisms by instantaneously transmitting isostatic pressure **(Bala et al., 2008; Oey et al., 2008)**. Conventional conservational processes use either physical or chemical means and thus they influence sensoric properties of raw materials and content of bioactive substances **(VSCHT, 2014)**.

HPP has been studied in several fruit juices, such as blueberry juice **(Barba, et al., 2013)**, tomato puree **(Rodrigo et al., 2007)**, apple juice **(Baron et al., 2006; Valdramidis et al., 2009)** and pomegranate juice **(Chen et al., 2013; Varela-Santos et al., 2012)**.

The aim of this work was to observe contents of important biologically active substances in fruit and vegetable juices and to compare their contents in fresh juice, in juice treated by pasteurization and pascalization.

MATERIAL AND METHODOLOGY

As the source of material to determine antioxidative activity, total polyphenols and flavonoids, 100% natural beverages, cold-pressed juices of one species or a mixture of different fruit or vegetable species, were used. They are without an addition of water, sugar, aroma or any other additives. Vegetable and fruit juices were treated by high pressure (600 MPa) and pasteurization (the temperature of 70 °C for 10 minutes) or they were used as fresh juice to compare with the processed juices. The samples were analyzed immediately after pressing in five repetitions.

The samples content was as follows: beetroot 100%, carrot 100%, broccoli with apple (apple juice 50%, broccoli juice 30%, orange juice 18%, lime juice 2%), cabbage with apple (apple juice 64 %, cabbage juice 32%, lemon juice 4%).

Total phenolic content assay

To measure total phenolic content (TPC) Folin-Ciocalteau reagent was used. 500 μL of the juice was taken and mixed with water in a 50 mL volumetric flask. Thereafter, 2.5 mL of Folin-Ciocalteau reagent and 7.5 mL of 20% solution of Na_2CO_3 were added. The resulting absorbance was measured by LIBRA S6 spectrophotometer (Biochrom Ltd., Cambridge, UK) at the wavelength of 765 nm. Water was used as reference **(Thaipong et al., 2006)**. The results were expressed as milligrams of gallic acid (GAE) per 100 mL.

Total flavonoid content assay

Total flavonoid content (TFC) was determined by using 300 mL of juice mixed with 3.4 mL of 30% ethanol, 0.15 mL of $NaNO_2$ (c = 0.5 mol.dm^{-3}) and 0.15 mL of $AlC1_3.6H_20$ (c = 0.3 mol.dm^{-3}) as is described by **Park et al., (2008)**. The mixture was measured at the wavelength of 506 nm by LIBRA S6 spectrophotometer (Biochrom Ltd., Cambridge, UK). Total flavonoid content was calculated from a calibration curve by using rutin as the standard. The results were expressed milligrams of rutin per 100 mL.

Total antioxidant capacity assay

determine total antioxidant capacity (TAC) DPPH (1,1-diphenyl-2picrylhydrazyl) assay was used according to the study by **Brand-Williams et al. (1995)**. The stock solution was prepared by dissolving 24 mg of DPPH with 100 mL of methanol and then stored at -20 °C until needed. The absorbance of DPPH radical without juice was measured daily. The sample solution was obtained by mixing 10 mL of the stock solution with 45 mL of methanol to obtain the absorbance of 1.1 ±0.02 units at 515 nm using the spectrophotometer LIBRA S6 (Biochrom Ltd., Cambridge, UK). The juice (150 μL) was allowed to react with 2.850 μL DPPH solution for 1 hour in the dark. Then, the absorbance was taken at 515 nm. Antioxidant capacity was calculated as a decrease of the absorbance value using the formula:

Antioxidant capacity (%) = (A0 - Ai/A0) x 100%, where A is the absorbance of a blank (without the sample) and Ai is the absorbance of the mixture containing the sample. Calculated antioxidant capacity was converted using a calibration curve of the standard and expressed in ascorbic acid equivalents (AAE) **(Rupasinghe et al., 2006)**.

Statistical analysis

The data were analyzed using Adstat v.1.25 (TriloByte) and expressed as means ± standard deviations. Any significant differences between samples were determined by one-way analysis of variance, considering differences significant at p <0.05. This statistical analysis was performed with Statistica v.1.25 (StatSoft).

RESULTS AND DISCUSSION

Antioxidant capacity

Results of the amounts of antioxidant capacity are shown in Table 1.

Antioxidative activity in fresh juice of broccoli and apple was found to be 189.12 ±4.2 mg.100 mL^{-1}. It decreased after the thermal treatment by pasteurization from 189.12 ±4.2 mg.100 mL^{-1} to 170.87 ±5.21 mg.100 mL^{-1}. Pasteurization treatment caused an expected fall on antioxidative activity. However, after pascalization, treatment by high pressure, antioxidative activity rose 189.12 ±4.2 mg.100 mL^{-1} to 217.12 ±7.26 mg.100 mL^{-1}. Therefore the impact of pascalization on antioxidative capacity is positive.

Antioxidative activity in fresh juice of cabbage with apple was determined in the value of 163.32 ±2.65 mg.100 mL^{-1}. After the heat treatment by pasteurization antioxidative activity declined from 163.32 ±2.65 mg.100 mL^{-1} to 159.11 ±4.23 mg.100 mL^{-1}. An anticipated drop in antioxidative activity manifested again. Surprisingly, pascalization induced a relatively distinctive decrease in antioxidative activity compared with fresh juice, specifically from 163.32 ±2.65 mg.100 mL^{-1} to 109.54 ±5.65 mg.100 mL^{-1}.

Antioxidative activity of fresh beetroot juice was 168.54 ±7.84 mg.100 mL^{-1}. It remained at almost the same value as in fresh juice after pasteurization, specifically at 168.01 ±5.26 mg.100 mL^{-1}. After the treatment of pascalization, it increased from 168.54 ±7.84 mg.100 mL^{-1} to 186.77 ±9.45 mg.100 mL^{-1} which could be considered as a positive result of the experiment.

In fresh carrot juice antioxidative activity was 37.24 ±4.23 mg.100 mL^{-1} which is quite lower in comparison with other juices. After the heat treatment it rose from 37.24 ±4.23 mg.100 mL^{-1} to 43.14 ±0.56 mg.100 mL^{-1} compared with fresh juice. A slight decrease of antioxidative activity from 37.24 ±4.23 mg.100 mL^{-1} to 36.78 ±5.26 mg.100 mL^{-1} was caused by pascalization.

The treatment of pascalization should lead to maintenance of antioxidative activity within the examined matter while pasteurization should cause a decrease of antioxidative activity in comparison with an unprocessed sample.

It has been proved also by already quoted references. **Patras et al. (2009a)** established a higher antioxidative activity in the sample treated by HPP than in the sample after the heat treatment in strawberry and blackberry puree. **McInerney et al. (2007)** studied the activity and other parametres in vegetable. It has been proved that antioxidative activity has not changed after pascalization. In other studies **Patras et al. (2009b)** observed antioxidative activity in tomato and carrot puree. The

Table 1 Determination of antioxidative capacity in fresh and treated beverages.

Antioxidative capacity (DPPH assay) (mg.100 mL^{-1})			
	Fresh juice	**Pasterization**	**Pascalization**
Broccoli with apple	189.12 ±4.2^{a}	170.87 ±5.21^{b}	217.12 ±7.26^{c}
Cabbage with apple	16.,32 ±2.65^{a}	159.11 ±4.23^{a}	109.54 ±5.65^{b}
Beetroot 100%	168.54 ±7.84^{a}	168.01 ±5.26^{a}	186.77 ±9.45^{b}
Carrot 100%	37.24 ±4.23^{a}	43.14 ±0.56^{b}	36.78 ±5.26^{a}

The different superscripts in rows indicate statistically significant differences between data groups (statistically tested on level of significance α = 0.05).

Table 2 Determination of total polyphenols in fresh and processed beverages.

Total polyphenols (mg.100 mL^{-1})			
	Fresh juice	**Pasteurization**	**Pascalization**
Broccoli with apple	174.55 ±7.23^{a}	142.97 ±8.14^{b}	183.74 ±4.21^{c}
Cabbage with apple	105.12 ±4.21^{a}	102.32 ±3.33^{a}	114.84 ±6.54^{b}
Beetroot 100%	126.41 ±1.21^{a}	95.47 ±2.39^{b}	154.1 ±4.02^{c}
Carrot 100%	35.2 ±0.15^{a}	26.87 ±0.89^{b}	33.32 ±2.04^{ab}

The different superscripts in rows indicate statistically significant differences between data groups (statistically tested on level of significance α = 0.05).

activity was even significantly higher within the matter treated by high pressure (400-600 MPa) than in the samples without any processing or after the heat treatment. Similar conclusions are brought by **Chen et al. (2015)** stating a slight rise of antioxidant capacity after HPP and decrease after the heat treatment in papaya beverage.

Total phenolic content

The values of total phenolic contents are shown in Table 2.

In fresh juice of broccoli with apple the content of polyphenols was 174.55 ±7.23 mg.100 mL^{-1}. Polyphenolic content decreased after the heat treatment of pasteurization from 174.55 ±7.23 mg.100 mL^{-1} to 142.97 ±8.14 mg.100 mL^{-1} which responds to literature data. The treatment of high pressure, pascalization, caused an increase of polyphenol content from to 183.74 ±4.21 mg.100 mL^{-1} which brings a positive contribution of the experiment.

Polyphenolic content in fresh juice made from cabbage with apple was 105.12 ±4.21 mg.100 mL^{-1}. It slightly fell from 105.12 ±4.21 mg.100 mL^{-1} to 102.32 ±3.33 mg.100 mL^{-1} after the heat treatment which had been anticipated. The treatment of high pressure induced a growth of polyphenolic content from 105.12 ±4.21 mg.100 mL^{-1} to 114.84 ±6.54 mg.100 mL^{-1}.

Polyphenolic content in beetroot fresh juice was 126.41 ±1.21 mg.100 mL^{-1}. It fell after the heat treatment of pasteurization to 95.47 ±2.39 mg.100 mL^{-1}. The treatment by high pressure caused an increase in polyphenolic content from 126.41 ±1.21 mg.100 mL^{-1} to 154.1 ±4.02 mg.100 mL^{-1} which has proved the hypothesis.

Polyphenolic content in carrot fresh juice was determined to be 35.2 ±0.15 mg.100 mL^{-1}. Pasteurization reduced it from 35.2 ±0.15 mg.100 mL^{-1} to 26.87 ±0.89 mg.100 mL^{-1}. Pascalization caused a slight fall in polyphenolic content to 33.32 ±2.04 mg.100 mL^{-1}. While it was still a higher amount than the value after the heat treatment, it may be considered as positive.

Pascalization should maintain the amount of polyphenolic content in processed material, while pasteurization could cause the fall of polyphenolic content when compared with non-processed matter which is also stated by **Patras et al. (2009a)**.

The expectation of the fall of polyphenolic content after the heat treatment has been proved by this study. **Chipurura et al. (2010)** confirmed the decline of polyphenolic content after the heat treatment as well.

Even though a slight decrease of polyphenolic content in carrot juice was performed after pascalization, there was a growth of polyphenolic content in all the other tested samples. **Patras et al. (2009b)** examined carrot and tomato puree after the heat treatment and pascalization. Regarding the heat treatment, the fall of polyphenolic content was measured in both carrot and tomato puree. The value of polyphenolic content after HPP depended on the applied pressure. In carrot puree it declined with a growing pressure. With the pressure between 400 and 500 MPa the content was higher than in fresh juice and it slightly dropped with the applied pressure higher than 600 MPa. In tomatoes it grew with the increasing pressure. Compared

Table 3 Determination of total flavonoids in fresh and processed beverages.

	Total flavonoids (mg.100 mL^{-1})		
	Fresh juice	**Pasteurization**	**Pascalization**
Broccoli with apple	40.71 ±0.15^{a}	45.14 ±0.08^{b}	30.21 ±0.54^{c}
Cabbage with apple	7.21 ±0.04^{a}	5.69 ±0.56^{b}	6.53 ±0.99^{ab}
Beetroot 100%	45.25 ±2.14^{a}	43.98 ±1.02^{a}	36.77 ±0.36^{b}
Carrot 100%	9.74 ±0.14^{a}	6.65 ±0.09^{b}	5.29 ±0.09^{c}

The different superscripts in rows indicate statistically significant differences between data groups (statistically tested on level of significance α = 0.05).

with fresh juice, it was lower at the pressure of 400 MPa and higher at 500 MPa.

Chen et al. (2015) brought similar conclusions with papaya beverage performing a slight growth in total polyphenols after HPP and a fall after the thermal treatment. On the other hand, **Barba et al. (2010)** stated that no significant changes in total phenolics were observed between HHP treated and thermally processed vegetable beverage and unprocessed beverage.

Total flavonoid content

The results of total flavonoids are shown in Table 3.

Flavonoid content in fresh juice from broccoli with apple was 40.71 ±0.15 mg.100 mL^{-1}. It surprisingly rose to 45.14 ±0.08 mg.100 mL^{-1} after the heat treatment. Pascalization caused a lower flavonoid content of 30.21 ±0.54 mg.100 mL^{-1} which was a significant reduction.

Flavonoid content in fresh juice made from cabbage with apple was 7.21 ±0.04 mg.100 mL^{-1}. It decreased to the value of 5.69 ±0.56 mg.100 mL^{-1} after the heat treatment. Pascalization caused a smaller decline of flavonoid content to the level of 6.53 ±0.99 mg.100 mL^{-1}. In fresh beetroot juice flavonoid content was found to be 45.25 ±2.14 mg.100 mL^{-1}. It expectedly fell to the value of 43.98 ±1.02 mg.100 mL^{-1} after the heat treatment of pasteurization. Pascalization, technology using high pressure, induced a significant decline in flavonoids to the amount of 36.77 ±0.36 mg.100 mL^{-1} albeit a smaller decline was expectable due to a gentle processing method of pascalization.

Flavonoid content in fresh carrot juice was determined to be 9.74 ±0.14 mg.100 mL^{-1}. Not surprisingly, it decreased to the amount of 6.65 ±0.09 mg.100 mL^{-1}. Similarly as in the experiment with the beetroot juice, flavonoid content also fell after pascalization to the value of 5.29 ±0.09 mg.100 mL^{-1}.

Pascalization processing should result in the maintenance of flavonoid content while after pasteurization a drop in flavonoid content is expected in comparison with unprocessed material. It has been supported by many studies, also by **Chen et al. (2011)** who examined phenolic and flavonoid content and antioxidative activity in soya milk. After pascalization higher flavonoid content was reached than in fresh or in heat-treated matter **(Chen et al., 2011)**.

As flavonoids belong to the group of polyphenolic compounds, it could be expected to gain similar measured results as within total polyphenols. It may be due to a higher sensitivity of flavonoid fraction for high pressure treatment.

CONCLUSION

Determination of antioxidative activity by DPPH assay has shown a decline in the activity in most of the samples after the heat treatment. Pascalization led to the growth of antioxidative activity in broccoli and beetroot juice, in carrot juice the activity was lower even though it was a little bit higher than in pasteurized juice. In juice made from cabbage with apple antioxidaive activity decreased after pascalization as well.

Regarding polyphenolic content in studied samples, it decreased after pasteurization in all the samples without an exception. Pascalization caused a slight drop in polyphenolic content in carrot juice and an increase in all other juices.

Flavonoid content in fruit and vegetable juices was higher after pasteurization in juice from broccoli with apple. In the other samples it decreased. After the treatment by high pressure the amount of flavonoids fell a little in cabbage and apple juice. In the other samples it fell more significantly than after pasteurization.

With the exception of flavonoids, hypotheses stating that HPP is a gentle conservation method enabling maintaining a higher amount of bioactive and antioxidative substances than the heat treatment.

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Contact address:

Jiří Mlček, Tomas Bata University in Zlin, Faculty of Technology, Department of Food Analysis and Chemistry, Vavreckova 275, 760 01 Zlin, Czech Republic, E-mail: mlcek@ft.utb.cz.

Tünde Juríková, Constantine the Philosopher University in Nitra, Faculty of Central European Studies, Institut for teacher training, Drazovska 4, 949 74 Nitra, Slovakia, E-mail: tjurikova@ukf.sk.

Soňa Škrovánková, Tomas Bata University in Zlin, Faculty of Technology, Department of Food Analysis and Chemistry, Vavreckova 275, 760 01 Zlin, Czech Republic, E-mail: skrovankova@ft.utb.cz.

Marcela Paličková, Tomas Bata University in Zlin, Faculty of Technology, Department of Food Analysis and Chemistry, Vavreckova 275, 760 01 Zlin, Czech Republic, E-mail: marpali@gmail.com.

Jana Orsavová, Tomas Bata University in Zlin, Language Centre, nam, T. G. Masaryka 5555, Zlin, Czech Republic, E-mail: jana.orsavova@gmail.com.

Ladislava Mišurcová, Tomas Bata University in Zlin, Faculty of Technology, Department of Food Analysis and Chemistry, Vavreckova 275, 760 01 Zlin, Czech Republic, E-mail: misurcova@ft.utb.cz.

Irena Hlaváčová, Tomas Bata University in Zlin, Faculty of Technology, Department of Food Analysis and Chemistry, Vavreckova 275, 760 01 Zlin, Czech Republic, E-mail: hlavacovai@ft.utb.cz.

Jiří Sochor, Mendel University in Brno, Faculty of Horticulturae, Department of viticulture and enology, Valtická 337, 691 44 Lednice, Czech Republic, E-mail: sochor.jirik@seznam.cz.

Daniela Sumczynski, Tomas Bata University in Zlin, Faculty of Technology, Department of Food Analysis and Chemistry, Vavreckova 275, 760 01 Zlin, Czech Republic, E-mail: sumczynski@ft.utb.cz.