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EFFECT OF THERMAL PASTEURIZATION AND HIGH PRESSURE PROCESSING ON BIOACTIVE PROPERTIES IN STRAWBERRY JUICE

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ABSTRACT

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In the current food industry, companies often offer new and revolutionary processing methods that allow to improve food properties. A prominent technology is High Pressure Processing (HPP), a non-thermal technology that arises as an alternative to the traditional thermal pasteurization (TP). With HPP it is possible to obtain food and drinks similar to the raw food while improving important nutritive and functional properties. Since strawberries are very important fruit in the human diet, the aim of this study was to study the effect of HPP and TP on selected qualitative-quantitative parameters of strawberry juices (HPSJ - High Pressure Strawberry Juice/TPSJ - Thermal Pasteurized Strawberry Juice). It seems that strawberries can have a positive effect on human health due to their high content in beneficial nutrients. From monitored parameters, significant differences (p < 0.001) were found between juices in the following parameters: antioxidant activity, β -carotene and zeaxanthin content. Higher antioxidant activity (1547.60 ±4.89 mg AA.L⁻¹ FM vs. 1424.72 ±10.66 mg AA.L⁻¹ FM) and zeaxanthin (1.34 ±0.11 µg.mL⁻¹ FM vs. 0.89 ±0.08 µg.mL⁻¹ FM) was found in HPSJ, comparatively to TPSJ. The content of β -carotene was higher in TPSJ (156.28 ±2.13 µg.mL⁻¹ FM) than in HPSJ (122.02 ±4.28 µg.mL⁻¹ FM). Results related to the polyphenols content showed significantly higher values (p > 0.01) in HPSJ, compared to TPSJ (1100.04 ±17.16 mg GAE.L⁻¹ FM vs. 1002.66 ±17.16 mg GAE.L⁻¹ FM). The difference in the content of lutein (TPSJ 8.84 ±0.57 µg.mL⁻¹ FM; HPSJ 8.17 ±0.13 µg.mL⁻¹ FM) was not significant (p > 0.05).

Keywords: strawberry; thermal pasteurization (TP); high pressure processing (HPP); juice

INTRODUCTION

Cardiovascular disorders (CVD) are one of the leading causes of death and disability in the world (Bansilala et al., 2015; Protulipac et al., 2015) and their prevention is a major public health challenge (Perk et al., 2012). The oxidative modification of the Low-density lipoprotein (LDL) in the vascular wall seems to be a key factor in the development of atherosclerosis that is considered as the main cause of CVD (Wilson et al., 1998). A lower incidence of chronic diseases seems to be linked to the consumption of flavonoid-rich foods, particularly fruits and vegetables (Dragsted et al., 2006). Their consumption is associated with positive effects in prevention of the cardiovascular diseases (Takachi et al., 2010), ischemic stroke (Joshipura et al., 1999), cancer (Peterson et al., 2003; Riboli et al., 2003; Bosetti et al., 2005; Reis et al., 2012), diabetes mellitus - type 2 (Carter et al., 2010), metabolic syndrome (Esmaillzadeh et al., 2006) and other diseases. These foods often show anti-inflammatory effects (Larrosa et al., 2010), are normolipidemic and normo-glicemic agents (Chong et al., 2010; Tarozzi et al., 2010), being beneficial against age-related diseases (Halliwell, 2008), and have a significant role in eye health (Kalt et al., 2010). All these effects are related to the inhibition of LDL oxidation (Cook and Samman, 1996).

Besides whole fruits and vegetables, a relevant part of the intake of antioxidant coumpounds such as polyphenolic

phytochemicals (including anthocyanins) is supplied by fruit juices, since they are suitable food products in terms of ingestion of health-protective phytochemicals. Bioactive components may even be better absorbed from juices than from plant tissues. Some studies have shown that the intake of berry juices can increase the plasma antioxidant capacity, which suggests an improvement of the antioxidant status and indicates that antioxidant constituents of the juice might decrease lipid oxidation within plasma compartment (Jakobek et al., 2007).

Generally, manufacturers use thermal pasteurization (TP) in the production of juices in order to prevent their microbial spoilage and thus extend the shelf-life. However, it has been shown that TP induces deleterious changes in sensorial and nutritional quality of the processed juices. Furthermore, nowadays, the modern consumers demand minimally processed, high quality healthy drinks, with a fresh taste, free from additives and with the same or more nutrients than the natural raw products (**Considine et al. 2008**).

High pressure processing (HPP) is an industrially tested technology that offers a more natural, environmentally friendly alternative to the traditional TP for a wide range of food products (**Barbosa-Cánovas et al., 2005**). Nonthermal technologies are useful, not only to inactivate microorganisms and enzymes, but also to improve extractions' yield of desirable compounds, develop new

ingredients and market foods with novel quality and nutritional characteristic (Tokuşoğlu and Swanson, 2015). HPP, operating at room or refrigeration temperature, is an attractive innovative technology, which involves using cold isostatic high hydraulic pressure (ranges from 100 – 800 MPa) (Xi, 2006). The use of lower temperatures allows a better retention of the food nutritional components as well as their sensory characteristics attributed to "fresh" or "just prepared," (Barbosa-Cánovas et al., 2005), which may lead to the development of new products with novel functional properties (Mota et al., 2013). In high pressure extraction (HPE) process, the cell membranes are destroyed by the use of elevated pressures which leads to a greater number of bioactive compounds extracted out of the cells (Joo, et al., 2013). As a result, HPP has become a post-packaging convenient technology for foods whose quality would otherwise be altered by TP. Moreover, HPP can add a significant shelf-life increase to an existing refrigerated product. In fact, it has the potential to deliver chemical- or additive-free products with minimum impact on shelf life (Barbosa-Cánovas et al., 2005).

The aim of this work was to verify and compare the effects of two different processing technologies on strawberry juices's antioxidant activity, polyphenols, and carotenoid content (β -carotene, zeaxanthin and lutein).

MATERIAL AND METHODOLOGY

Biological material: Both juices (HPSJ and TPSJ) were prepared without the addition of preservatives and sweeteners, and were not made from concentrate (in ratio 80 - 85 % strawberries: 10 - 15 % water). The origin of strawberries was Portugal and Spain and the juice was kindly supplied by Sumol +Compal (Portugal). Two different processing methodologies were used: in the first one the strawberry juice was processed by HPP (HPSJ -High Pressure Strawberry Juice), whereas in the second it was processed by TP (TPSJ - Thermal Pasteurized Strawberry Juice). The used processing conditions in the HPSJ and TPSJ were the following: 550 MPa, at 20 °C for 2 min and 95 ±2 °C for 1 min, respectively.

TP was performed by the suplier of the juices and HPP was performed at the University of Aveiro. The processed strawberry juices were transported from the University of Aveiro in Portugal to Slovakia in refrigerated conditions. All analyses were realized in the Slovak University of Agriculture in Nitra.

Preparation of the sample: 10 g of the homogenized samples was transferred into a 100 mL Erlenmeyer flask, mixed with 70 mL of distilled water and extracted at a speed of 150 min⁻¹/24 hours. After 24 hours, it was added distilled water to make 100 mL, filtered and used for analysis. The extraction of both juices (HPSJ and TPSJ) was carried out in the same manner for the analysis.

Determination of antioxidant activity: The antioxidant activity in the strawberry juices (both HPSJ and TPSJ) was determined by the FOMO method (**Prieto et al., 1999**). The principle of the method is the reduction of Molybdenum (VI+) to Molybdenum (V+) by the activity of the reducing component in the phosphorus presence. There is a green Phosphomolybdic complex which color intensity is measured at a wavelength of 695 nm by spectrophotometer. The reductive ability of compounds

can be expressed as ascorbic acid (AA) content, which is needed to achieve the same reduction effect. The antioxidant activity was expressed in $mg.L^{-1}$ equivalent of ascorbic acid (AA).

Determination of polyphenols: The polyphenols of juices HPSJ and TPSJ was determined by spectrophotometry at a wavelength of 700 nm using the Folin-Ciocalteu method (**Singleton and Rossi, 1965**) and was measured as the equivalent content of gallic acid and expressed in mg GAE.L⁻¹ (gallic acid equivalent). The method is based on the reaction of the Folin–Ciocalteu reagent with polyphenols, with leads to the formation of a blue color product. The intensity of the blue color is proportional to the content of the polyphenols.

Determination of carotenoids: The carotenoid content expressed in β -carotene, zeaxanthin and lutein of the HPSJ and TPSJ juices was measured at a wavelength of 445 nm using a spectrophotometer by method of liquid chromatography HPLC (HPLC Agilent Infinity 1260) with slight modification, as described by Lachman et al. (2013) and Ashokkumar et al. (2015).

The results were processed by the statistical package STATISTICA Cz version 10. The differences between the samples were followed by Tukey's HSD test.

RESULTS AND DISCUSSION

Consumers around the world are better educated and more demanding in their identification and purchase of quality health-promoting foods. The food industry and regulatory agencies are searching for innovative technologies to provide safe and stable foods for their clientele. Thermal pasteurization and commercial sterilization of foods provide safe and nutritious foods that, unfortunately, are often heated beyond a safety factor that results in unacceptable quality and nutrient retention. (Tokuşoğlu and Swanson, 2015). Thermal degradation of anthocyanins results in the formation of polyphenolic degradation products; it is not clear if the formation of these components results in an overall reduction in antioxidant activity (Patras et al., 2010). Non-thermal processing technologies offer unprecedented opportunities and challenges for the food industry to market safe, highquality health-promoting foods. These technologies for food processing provide an excellent balance between safety and minimal processing, between acceptable economic constraints and superior quality, and between unique approaches and traditional processing resources (Tokuşoğlu and Swanson, 2015).

The health benefit of fruit juices has been described, in part, to natural antioxidants which may inhibit the development of major clinical conditions including cardiovascular disease and cancer. Many fruit juices also contain phenolic compounds and carotenoids which have antioxidant potential (Gardner et al., 2000). Antioxidant activity is associated with decreases in DNA damage, reduction in lipid peroxidation, maintenance of the immune function, and prevention of the development of some diseases (Gropper, Smith and Groff 2005). The function of β -carotene and carotenoids is manifested in the antioxidant activity, inhibition of lipid peroxidation and increased resistance of LDL to oxidation, the antioxidant protection against sunlight. Zeaxanthin affects visual acuity, is important for immunity, intercellular

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Processing method	Antioxidant activity (mg AA.L ⁻¹ FM)	Polyphenols (mg GAE.L ⁻¹ FM)	<mark>β-carotene</mark> (μg.mL ⁻¹ FM)	Zeaxanthin (μg.mL ⁻¹ FM)	Lutein (µg.mL ⁻¹ FM)
HPSJ	1547.60 ±4.89	1100.04 ± 17.16	122.02 ± 4.28	$1.34\pm\!\!0.11$	8.17 ±0.13
TPSJ	1424.72 ± 10.66	1002.66 ± 17.16	156.28 ±2.13	0.89 ± 0.08	8.84 ± 0.57
<i>p</i> -value	0.001	0.01	0.001	0.001	0.062
Significance	++	+	++	++	-

Table 1 Statistical evaluation of average values of monitored parameters in strawberry juices.

Note: Data are expressed as mean $\pm SD$ (standard deviation); ANOVA, Tukey's HSD test; FM – fresh mater.

communication and metabolism of arachidonic acid (Keresteš et al., 2016). β -carotene preventively protects against cardiovascular diseases, lung cancer and other cancers of the gastrointestinal tract (Nemeth-Balogh et al., 2009).

Strawberries are the most popular berries in the world, being a nutritious fruit with putative health benefits (Hossain et al., 2016; Giampieri et al., 2012). Bioactive components and anthocyanin compounds of strawberries have antioxidant, anti-inflammatory, antihypertensive, antihyperlipidemic and antiproliferative effects (Basu et al., 2014). Studies showed that strawberries have high concentrations of ascorbic acid (Larson, 1998; Szajdek and Borowska, 2008), anthocyanins (da-Silva et al., 2007), phenolic acids, flavonoids, vitamins, carotenoids (Kelebek and Selli, 2011), ellagic acid (Häkkinen et al., 2000), and can achieve high total antioxidant capacity (Wang and Lin, 2000).

We found that the HPSJ presented significantly (p < 0.001) higher antioxidant activity (1547.60 ±4.89 mg AA.L⁻¹ fresh matter) than TPSJ (1424.72 ±10.66 mg AA.L⁻¹ fresh matter). With the same method, **Mendelová et al., (2016)** found that antioxidant activity of sea buckthorn fruit juice ranged from 45.11 g AA.L⁻¹ dry matter to 108.77 g AA.L⁻¹ dry matter.

The results showed a significant (p < 0.01) higher amount of total polyphenols in the HPSJ (1100.04 ±17.16 mg GAE.L-1 fresh matter) than the in TPSJ $(1002.66 \pm 17.16 \text{ mg GAE}.L^{-1} \text{ fresh matter})$. In another study, the polyphenols conten ranged from 532 to 960 µg GAE.mL⁻¹ in fruit citrus juice (**Rekha et al., 2012**). According to Rolle et al., (2016) concentrations of polyphenols were similar between the two types of orange juices, the values of commercial thermally pasteurized juice was $63.3 \pm 5.85 \text{ mg}.100 \text{mL}^{-1}$ and home-made fresh juices were $62.9 \pm 5.94 \text{ mg}.100 \text{mL}^{-1}$.

Statistically, significant differences (p < 0.001) were reported in the content of β -carotene and zeaxanthin between the studied juices. The difference in the content of lutein in the juice HPSJ and TPSJ was not statistically confirmed (p > 0.05). The content of β -carotene was 156.28 ±2.13 µg.mL⁻¹ fresh matter in TPSJ, whereas in HPSJ was found a lower value 122.02 ±4.28 µg.mL⁻¹ fresh matter.

In studies focused on the investigation of these ingredients in different kinds of vegetables, fruits, juices and other food products, they were reported different values, which are associated with inconsistent methodologies: β -carotene's content in carrots was 192.25 ±19.70 µg.g⁻¹ dry matter (**Stinco et al., 2014**), and **Maurer et al., (2014**) indicated the amount of 53.6 µg.g⁻¹ fresh matter. Different amounts of β -carotene was found in apples (0.17 µg.g⁻¹ dry matter) (**Delgado-Pelayo et al., 2014**), canned apricots (170.0 µg.g⁻¹ fresh mater) and canned peaches (9.3 µg.g⁻¹ fresh matter) (**Campbell and Padilla-Zakour, 2013**).

On the contrary, the content of zeaxanthin was $0.89 \pm 0.08 \ \mu g.m L^{-1}$ fresh matter in TPSJ, whereas in HPSJ a higher value was found $(1.34 \pm 0.11 \ \mu g.m L^{-1}$ fresh matter). Also, the Zeaxanthin content found in different fruits was variable: in apples was $0.01 \ \mu g.g^{-1}$ dry matter (**Delgado-Pelayo et al., 2014**), in canned apricots 2.7 \ \mu g.g^{-1} and in canned peaches 1.1 \ \mu g.g^{-1} fresh matter (**Campbell and Padilla-Zakour, 2013**).

The content of lutein was higher in TPSJ than HPSJ (8.84 $\pm 0.57 \ \mu g.mL^{-1}$ fresh matter and 8.17 $\pm 0.13 \ \mu g.mL^{-1}$ fresh matter, respectively). The lutein content found in carrots by **Maurer et al.**, (2014) was 1.5 $\ \mu g.g^{-1}$ fresh matter, whereas **Stinco et al.**, (2014) reported values of 44.11 $\pm 6.11 \ \mu g.g^{-1}$ dry matter. **Delgado-Pelayo et al.**, (2014) reported that red apples contain 0.06 $\ \mu g.g^{-1}$ dry matter of lutein and canned apricots 0.09 $\ \mu g.g^{-1}$ fresh matter of lutein (Campbell and Padilla-Zakour, 2013).

Other studies evaluated the carotenoid content in a variety of fruits and juices. According to Aschoff et al., (2015), the concentration of β -carotene in orange juice was 11.1 ± 0.9 µg.100 g⁻¹, lutein 58.9 ± 0.8 µg.100 g⁻¹, and zeaxanthin 47.0 \pm 1.4 µg.100 g⁻¹, whereas the fresh oranges that were used for the preparation of the juice had higher values (β -carotene 21.1 ±1.6 µg.100 g⁻¹, lutein $61.2 \pm 1.6 \ \mu g.100 \ g^{-1}$ and zeaxanthin 49.4 $\pm 3.6 \ \mu g.100 \ g^{-1}$). The same tendency was observed in the content of peach β-carotene and zeaxanthin of juice $(1.61 \pm 0.82 \ \mu g.g^{-1} \text{ and } 0.19 \pm 0.02 \ \mu g.g^{-1})$ when compared to the fresh peaches $(1.98 \pm 0.62 \ \mu g.g^{-1})$ and 0.27 ±0.12 μg.g⁻¹) (Giuffrida, 2013). Esteve et al., (2009) reported that high pressure technology supports a higher extraction of biologically valuable substances of fruits and vegetables because they found higher levels of total carotenoids in orange juice treated by high pressure method (1309.2 \pm 46.7 µg.100 mL⁻¹) in comparison with the thermally pasteurized juice (1195.4±31.6 µg.100 mL⁻ ¹).

CONCLUSION

Based on the assessment of the qualitative-quantitative parameters, we found that there were statistically significant differences in the antioxidant activity, polyphenols and zeaxanthin content between HPSJ and TPSJ, where the former presented a clear advanteage. On the contrary, we found that TPSJ was able to retain higher values of β -carotene than HPSJ. Statistically, no significant differences (p > 0.05) were detected in the content of lutein in both juices.

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