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EFFECT OF PROCESSING ON VITAMIN C CONTENT, TOTAL PHENOLS AND ANTIOXIDATIVE ACTIVITY OF ORGANICALLY GROWN RED BEETROOT (*BETA VULGARIS* SSP. *RUBRA*)

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Abstract: The demand for organic food is rising since consumers want food from reliable, highest quality sources originating from the environment, undisturbed by cultivation and processing. It is necessary to determine to what extent there is a scientific basis for the claims that organic food is of high quality. In this study, beetroot from an organic production system originating from 6 certified organic food producers from different geographic locations was examined. The organic beetroot samples were processed by pasteurization at 70 °C and 90 °C into beet juice or by drying at 55 °C. The following samples were tested and compared: fresh beetroot, pasteurized beet juice and dried beetroot slices. The concentration of vitamin C, level of total phenol compounds (TPC) and antioxidative activity (TAA) in beetroot were influenced by the geographic origin and the applied processing method. The highest degradation for all analysed parameters was found in the samples treated by drying or pasteurisation at 90 °C. The lowest losses of studied phytochemical components were observed during juice pasteurisation at 70 °C. The correlation coefficient between TPC and TAA was high and significant (r^2 = 0.966).

Key words: *pasteurization, juice, drying, phytochemicals, degradation, geographic origin*

INTRODUCTION

In recent years there has been an increase in sales and consumption of organic as naturally aligned food. The trend of using natural resources in diet and notably food of plant origin increased along with new insights regarding their therapeutic impact on human health. Demands for organically produced food are high because consumers desire food from reliable sources, grown in environmentally friendly surroundings (air, soil, water) that guarantee food products of high quality (Bavec, Turinek, Grobelnik & Bavec, 2009; Burt et al., 2009; Domagała-Swiatkiewicz & Gastoł, 2012; Kazimierczak et al., 2014). The impact of farming systems on food quality and specifically on human health is very important (Reganold et al., 2010). In the past 20 years, many researchers dealt with the comparison of the food quality

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produced conventionally and organically. Many research studies proved that organically produced food products contain more bioactive components (vitamins and phenols), due to the prohibition of the use of synthetic pesticides and fertilizers (Bourn & Prescott 2002; Bavec, Mlakar, Rozman, Pazek & Bavec, 2009; Straus et al. 2012; Kazimierczak et al., 2014).

Plants can be an excellent source of phytochemical compounds with marked antioxidative and anticancer activity (Zein, Hashish & Ismaiel, 2015). The interest in the biological activity of beetroot keeps growing although it is well known as a health-promoting functional food. Beetroot is a traditional vegetable all over the world, much consumed in the western kitchen. Beetroot belongs to the family Chenopodiaceae (Ninfali & Donato, 2013). It is mainly grown in Europe, Asia, the USA and the Mediterranean (Lee, Wettasinghe, Bolling, Ji & Parkin, 2005). It is a very cheap and renewable source of nutrients. It is not very demanding when it comes to growing. It can be grown on soil poor in organic matters, with little light and insufficient quantities of water (Ninfali & Donato, 2013). Both leaves and roots are used in human nutrition, usually in the form of sour salad or juice.

Beetroot contains bioactive compounds beneficial for human health, such as vitamin C and E, phenols, flavonoids (Paganga, Miller & Rice-Evan, 1999; Georgiev et al., 2010; Clifford, Howatson, West & Stevenson, 2015). Some studies have provided unequivocal evidence that the beetroot has a positive effect on the clinical picture of the diseases such as hypertension, atherosclerosis, diabetes and dementia (Ninfali & Donato, 2013; Gilchrist et al., 2014). One of the powerful antioxidants in beet is vitamin C. It neutralizes free radicals in an organism and, generally, represents a central indispensable component for the whole body. However, it easily degrades during cooking (Njoku, Ayuk & Okoye, 2011). A great deal of antioxidative activity of vegetables, besides vitamin C, depends on phenolics content (Oboh & Rocha 2008). Beetroot is among those vegetables rich in phenolic acids, which directly contribute to its high antioxidative capacity. However, the processing method highly affects the phenolic acid content in processed beetroot (Ravichandran, Abdelrahman, Dietrich & Smetanska, 2012). Besides the use of fresh vegetables in the human diet, the use of vegetable juices is getting more popular. Thermal processing is necessary to prolong the shelf-life of juice and, consequently, the availability of bioactive components. The application of different food processing methods can have a different effect on the phytochemical composition of products (Guldiken et al., 2016). Thermal pasteurisation of fruit and vegetable juices is one of the most common methods for shelf-life prolongation by inactivation of microorganisms and enzymes responsible for decay (Kathiravan, Nadanasabapathi & Kumar, 2015).

Generally, it is believed that thermal processsing diminishes the nutritional quality of food. However, there are cases of increased food quality after thermal processing (Ravichandran et al., 2012). The main factors that influence the changes in food quality are the time and temperature of food processing (Guldiken et al., 2016). According to Madrau et al. (2009), the highest number of nutrients in food decrease with thermal processing at high temperatures. One of the oldest methods of conservation is drying. It can be traditional such as drying in the sun or hot air in different drier types (Gokhale & Lele, 2011; Jiokap, Aseaku, Desmorieux, Degraeve & Kamga, 2015). The advantage of dried against fresh vegetables are numerous: easier transport and usage, longer preservation (storage), etc. (Lewicki, Witrowa-Rajchert, Pomaranska-Lazuka & Nowak, 1998). Dried beetroot can be used as a snack or as a powder for the natural colouring of food (soups, sweets, gels, etc.) (Gokhale & Lele 2011). This research aimed to determine the level of vitamin C, total phenolics and total antioxidative activity in fresh and thermally processed beetroot originating from six different organic producers from Serbia. Thermal processing of organic beetroot included pasteurisation of juice at different temperatures (70 ºC and 90 ºC) and drying with hot air (50-55 ºC). The obtained results will improve the knowledge related to production of nutritionally satisfying beetroot juice.

MATERIALS AND METHODS

Plant material

The studied beetroot in this experiment belonged to type Bikor and was procured from an organic market in Belgrade by six different certified organic producers. Organic farms were from different parts of Serbia: Suvobor

(SB), Taras (TS), Gložanj (GL), Padina (PD), Kikinda (KI) and Kisač (KS). Fresh beetroot, thermally processed juice at 70 and 90 ºC and dried samples were analysed.

Beetroot was firstly washed, peeled and blended in a centrifugal juicer. The obtained juice was pasteurised in a water bath at 70 ºC and 90 ºC for 15 minutes. Dried samples of organic beetroot were prepared firstly by blanching at 100 ºC for 5 minutes, then peeling and cutting into tiny circular slices. The samples were dried in a home dehydrator at 50-55 ºC.

Determination of vitamin C

Pale beet juice was obtained by pressing 100 cm^3 of red beetroot juice and mixed with equal quantity of solution (100 cm^3) of a mixture of HPO_3 and glacial acid CH_3COOH. Then, the mixture was filtrated through creased filter paper. The first 5-10 cm^3 of filtrated mixture was thrown away and the aliquot part was taken from the rest of the mixture for the further investigation. If necessary, the investigated sample was diluted with cooled boiled distilled water, so the aliquot part contained about 2 mg of ascorbic acid. The process of determining ascorbic acid in the sample: 10 cm^3 of filtrated sample (containing 5 cm^3 of juice and 5 cm^3 HPO_3 and glacial acid CH_3COOH) was applied to three Erlenmeyer dishes using pipette. Each sample was titrated with Tillman's reagent (TR) solution until pale pink, for about 5 seconds. At the same time, solution of TR was titrated and blind tested until pale pink (Cvijović & Aćamović, 2005).

The content of ascorbic acid (mg/ cm^3) = $(V - V_1)$ x T x 100/g
V – cm^3 of TR solution used for titration in trial testing
V1 – cm^3 of TR solution used in blind testing
T – titre solution TR (mg $C_6H_8O_6$ /1 cm^3 TR solution)
g – juice volume in cm^3 in aliquot part of sample

Total phenolic content

Total phenols in beetroot ethanol extracts 20g beetroot juice, 100cm^3 in ethanol were estimated according to the Folin–Ciocalteu method (Singleton, Orthofer & Lamuela-Raventós, 1999). The extract was diluted to the concentration of 1 mg/mL, and aliquots of 0.5 mL were mixed with 2.5 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 mL of $NaHCO_3$ (7.5%). Aliquots were left for 15 minutes at 45 ºC, and then the absorbance was measured at 765 nm with a spectrophotometer against a blank sample. Gallic acid (GA) was used to calculate the standard curve. The assays were carried out in triplicate; the results were the mean values ± standard deviations and expressed as mg of gallic acid equivalents per gram of dry extract (mg of GA/g).

Total antioxidant activity

Determination of total antioxidant activity by DPPH method has been done spectrophotometrically (Xu et al. 2010). 8 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) were dissolved in methanol (100 mL) to give a concentration of 80 g/mL. Serial dilutions were made from the stock solution (1 mg/ml) of extract. Solutions (2 ml each) were then mixed with DPPH (2 mL) and allowed to stand for 30 minutes to any reaction occurred, and the absorption was measured at 517 nm. Ascorbic acid was used as the reference standard and dissolved in methanol to make a stock solution with the same concentration of 1 mg/ml. The control sample was prepared to contain the same volume, but without the test compound or reference antioxidants. 95% methanol was used as a blank. Three measurements were made.

Data analysis

Variations in the results due to different beet growers were calculated according to ANOVA model, and the significant difference was expressed by LSD test. Differences in the results coming from different processing procedures were expressed using Tukey's test. Correlative ratio among the traits according to researched samples from different localities was determined by applying the Pearson matrix at the significance level $P \leq 0.005$. The connection of samples and traits was done by multi-variation technique of PCA-Principal Component Analysis using statistical software XLSTAT Version 2012.4.02 Copyright Addinsoft 1995-2012. The analysis was performed according to average values of the researched parameters.

RESULTS AND DISCUSSION

Ascorbic acid (Vitamin C)

Vitamin C ($C_6H_8O_6$) is an l-enantiomer of ascorbic acid. Vitamin C or L-ascorbic acid belongs to a group of essential nutrients, necessary for the function of human organisms as well as other animal species (Njoku et al., 2011). Its main natural sources are fresh fruits and vegetables. This research showed that beetroot, as one of C vitamin's natural sources (especially if grown organically), contains 10.05-11.65 mg/100g vitamin C (Table 1). Szopinska and Gaweda (2013) found lower C vitamin content in beetroot and its content varied depending on a year and method of cultivation from 5.06 mg/100g to 9.46 mg/100g. Straus et al. (2012) reported high levels of vitamin C in beetroot ranging from 23.3 mg/100g to 33.9 mg/100g. Among the analyzed beetroot fresh samples originating from different geographic locations, vitamin C varied but was not statistically significant (*LSD* test, $p < 0.001$).

The concentration of vitamin C in fresh beetroot can vary depending on many factors such as cultivation, growing and climate conditions, geographic location, etc. (Leong & Oey, 2012; Szopinska & Gaweda, 2013; Kazimierczak et al., 2014). There was a statistically significant difference (Tukey's test; $p < 0.001$) in vitamin C between fresh beetroot and thermally processed juice at 90 ºC and dried samples (55 ºC). Interestingly, it was not observed a significant difference in vitamin C between fresh beetroot and juice thermally processed at 70 ºC (Table 1).

In this contest, there was no significant loss of vitamin C when cooking juice at 70 ºC. Our results are in line with Njoku et al. (2011), Adefegha and Oboh (2011), and Pavlovic et al. (2017) who found that the temperature of processing directly impacts the denaturation of vitamin C. Low temperatures provoke a high concentration of vitamin C in a product. Besides temperature, degradation of vitamin C is also affected by the duration of thermal treatment and pressure (Nemzer et al., 2011; Paciull, Medina-Meza, Chiavaro & Barbosa-Canovas, 2016).

Total phenolic compounds (TPC)

TPC are secondary plant metabolites. Many studies inferred their extraordinary importance in preservation of human health. The level of TPC in beetroot in this research varied from 39.05±0.15 mg GAE/g FW to 31.55±0.05 mg GAE/g FW (Table 2). Studies of other authors showed that the level of TPC in beetroot varied from 0.51 mg GAE/g FW (Bavec et al., 2010) to 255±48 mg GAE/100g sample (Guldiken et al., 2016). There was a statistically significant difference in TPC (*LSD* test, $p < 0.001$) among beetroots originating from 6 different organic farms (Table 2). Similar was noted by Leong and Oey (2012) who reported that location of the producer can impact the TPC content. In this study, it was found a statistically significant difference in TPC between fresh beetroot, juice (70 ºC and 90 ºC) and dried beetroot samples (Table 2).

Comparing the obtained results for TPC among fresh and thermally processed samples (juice and dried) it is clear that the level of TPC dropped during thermal processing (Table 2).

Processing such as cooking, drying under vacuum, etc. impacts the change of total phenols in food (Fang, Hu, Liu &Ye, 2008; Ravichandran et al., 2012; Guldiken et al., 2016; Pavlović et al., 2017).

Table 1.
Vitamin C content in differently processed red beetroot collected from various organic growers in Serbia

Beetroot samples	**Vitamin C content** (mg/100g FW)				**Tukey's test**	**Significance** P < 0.05; 0.01
	Fresh sample	**Pasteurised juice**		**Dried beet slices**		
		70 ºC	90 ºC			
SB	11.25	10.00	6.32	4.25	2.69	No
TS	10.26	9.45	5.43	3.03	14.33	Yes
GL	10.05	9.08	4.68	2.25	21.34	Yes
PD	11.43	10.25	6.75	4.31	11.64	Yes
KI	11.65	11.31	7.05	4.75	18.65	Yes
KS	11.00	10.12	6.55	4.05	7.02	Yes
$LSD_{0.05}$	1.718	1.133	0.56	0.392		
$LSD_{0.01}$	2.444	1.611	0.796	0.558		

Growing regions: Suvobor (SB), Taras (TS), Gložanj (GL), Padina (PD), Kikinda (KI) and Kisač (KS); FW-fresh weight

Table 2.
Total phenolics (TPC) content in red beetroot collected from various organic growers in Serbia as affected by different processing methods

Beetroot samples	**Total phenolics** (mg GAE/g FW)				**Tukey's test**	**Significant** P < 0.05; 0.01
	Fresh sample	**Pasteurised juice**		**Dried beet slices**		
		70 °C	90 °C			
SB	38.15±0.05	32.55±0.05	20.05±0.05	10.75±0.05	5.29	Yes
TS	39.05±0.15	33.25±0.10	21.45±0.05	11.32±0.05	19.18	Yes
GL	31.55±0.05	27.55±0.02	17.45±0.05	8.75±0.05	30.15	Yes
PD	34.25±0.25	31.07±0.05	19.07±0.05	9.75±0.05	13.90	Yes
KI	32.56±0.15	29.37±0.15	17.55±0.05	8.55±0.05	24.87	Yes
KS	35.05±0.15	30.22±0.05	18.50±0.05	9.75±0.05	10.97	Yes
$LSD_{0.05}$	0.773	1.548	0.924	1.048		
$LSD_{0.01}$	1.099	2.202	1.314	1.491		

Growing regions: Suvobor (SB), Taras (TS), Gložanj (GL), Padina (PD), Kikinda (KI) and Kisač (KS); FW-fresh weight

Table 3.
Total antioxidant activity (TAA) in red beetroot of various organic growers in fresh, dried and juice sample

Beetroot samples	**TAA** (mg AA/100 g FW)				**Tukey's test**	**Significance** P < 0.05; 0.01
	Fresh sample	**Pasteurized juice**		**Dried beet slices**		
		70 °C	90 °C			
SB	15.22±0.05	12.75±0.25	7.25±0.25	5.22±0.25	4.201	Yes
TS	15.45±0.15	13.05±0.25	6.78±0.05	5.70±0.05	18.59	Yes
GL	13.05±0.15	11.20±0.05	4.45±0.05	3.21±0.05	22.30	Yes
PD	14.75±0.05	13.25±0.15	6.65±0.05	3.25±0.05	14.39	Yes
KI	14.95±0.15	13.11±0.05	4.02±0.05	2.02±0.05	18.10	Yes
KS	13.78±0.25	11.98±0.15	5.56±0.05	4.85±0.05	3.705	No
$LSD_{0,05}$	1.544	0.979	0.852	0.487		
$LSD_{0,01}$	2.197	1.392	1.211	0.693		

Growing regions: Suvobor (SB), Taras (TS), Gložanj (GL), Padina (PD), Kikinda (KI) and Kisač (KS),FW-fresh weight

Total antioxidant activity (TAA)

Beetroot belongs to the group of vegetables with high antioxidative potential (Georgiev et al., 2010; Ravichandran et al., 2012) which is why it is highly appreciated in human diet. TAA level in this study in fresh, untreated beetroot samples was in the range from 13.05±0.15 mg AA/100 g to 15.45±0.15 mg AA/100 g (Table 3). TAA findings reported in other studies varied and depended on the applied method of analysis and expression of results (fresh weight (FW) i.e. dry matter (DM) basis), etc. Due to this fact, it is hard to compare results. Bavec et al. (2010) found that TAA in beetroot was from 0.823 µM TE/g (FW) do 1.270 µM TE/g (FW) (the TAA value was defined as the concentration of Trolox having equivalent antioxidant activity expressed as µM TE per gram fresh weight - µM TE/g FW). While Kugler, Stintzing and Carle (2007) reported the value of 11.103 µM TE/g. Comparing the beetroot samples originating from different farms (from mutually distant regions) in this research, it was concluded that there were statistically significant differences (*LSD* test, $p < 0.001$) among them. Variation in the content as well as the stability of bioactive components in plant material depending on the location of growers was reported in the study by Leong and Oey (2012). There was a statistically significant difference in TAA among pasteurised juice (70 ºC and 90 ºC) and dried beetroot samples (Table 3).

However, it was not found a statistically significant difference for TAA between the juice pasteurized at 90 ºC and the dried beetroot (Table 3). TAA of thermally processed beetroot decreased comparing to the fresh samples. The lowest TAA activity was observed in beetroot samples treated by drying (Table 3).

Our results are in line with those reported by Guldiken et al. (2016) who found that every thermal processing of beetroot decreases the

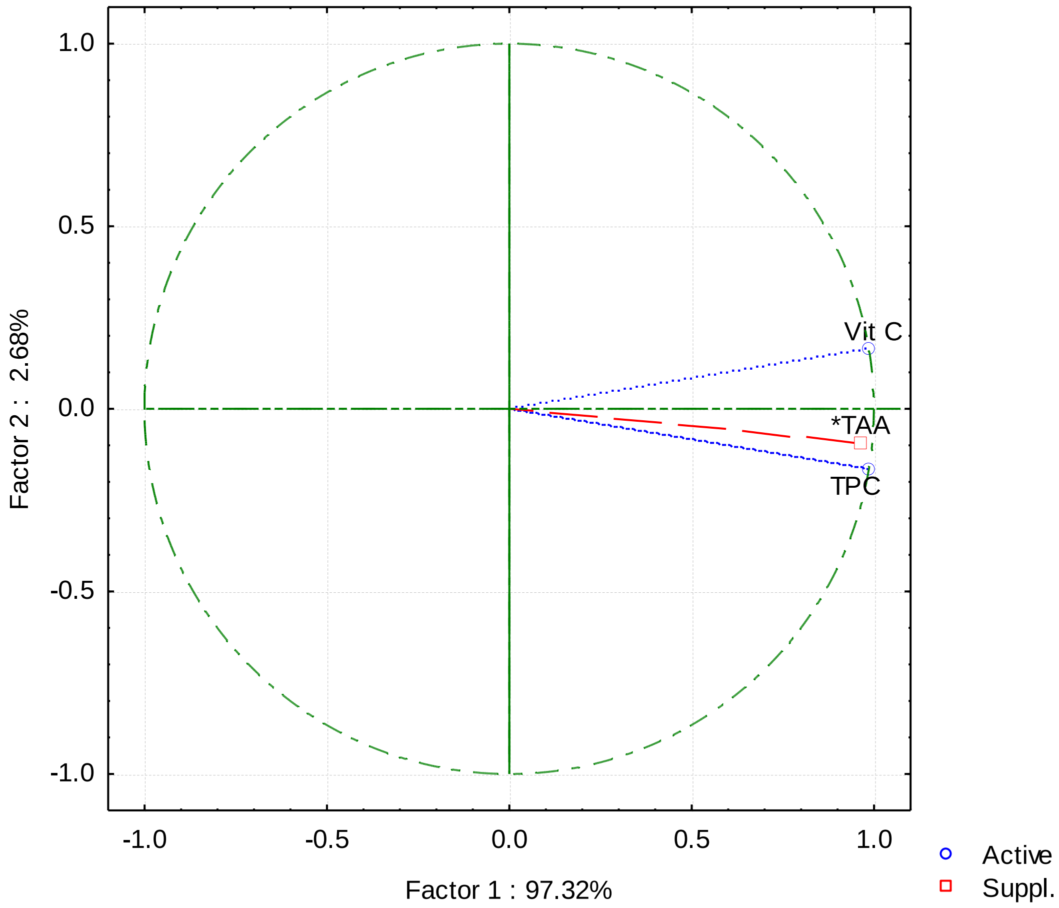

Figure 1. Pearson`s correlation figure of tested phytochemical components of red beetroot (Vitamin C content, total antioxidant activity (TAA), total phenolics (TPC)

antioxidative activity compared to fresh, untreated beet. Fang et al. (2008), experimenting with thermally processed mustard, came to the same conclusion. In contrast, Adefegha and Oboh (2011) found that cooking of plant material increases the antioxidative activity.

PCA analysis of variations in the dataset

Projection of variables according to Pearson correlation coefficient proves high positive statistically significant correlation among vitamin C and TPC with TAA (supplementary variable, Fig. 1). The highest level of correlation coefficient was calculated for correlation among TPC and TAA ($r^2 = 0.966$, $p < 0.005$).
Our findings are in accordance with results of Ravichandran et al. (2012), Georgiev et al. (2010), and Bavec et al. (2010). They confirmed high additive and synergistic impact of phytochemical compounds to TAA. They also found high correlation among TPC and antioxidative activity. In our study, the first principal component (PC_1) explained 97.32% of total variance. Contribution of the second principal (PC_2) to total variance was small 2.68% (Fig. 1).

CONCLUSIONS

This study showed that the level and stability of phytochemical components (vitamin C and TPC) and antioxidant activity (TAA) in beetroot during processing was influenced by the growing location and the way of processing. This study showed that thermal processing (either pasteurisation or drying) of thebeetroots reduced the content of phytochemical components and their activity relative to the fresh beetroot. The lowest losses were during the pasteurisation of juice at 700C. The highest degradation of vitamin C, TPC and TAA in all samples was after drying with hot air (55 ºC) followed by pasteurisation at 90 ºC.

There was a high correlation (r^2 = 0.966) between TPC and TAA for the tested samples. The obtained results in this study related to thermal pro-cessing of beetroot could impact the optimisation of similar processes to keep the best phytochemical quality of food.

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UTICAJ PRERADE NA SADRŽAJ VITAMINA C, UKUPNIH FENOLA I UKUPNU ANTIOKSIDATIVNOST U ORGANSKI PROIZVEDENOJ CVEKLI *(BETA VULGARIS* SSP. *RUBRA)*

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Sažetak: Potražnja za organskom hranom je u porastu jer potrošači žele hranu iz pouzdanih izvora gde proizvodni proces ne narušava životnu okolinu, a time se očekuje da hrana proizvedena u ovom sistemu bude na kvalitativno višem nivou. Potrebno je utvrditi u kojoj meri postoji naučna osnova za tvrdnje da je organska hrana visoko kvalitetna. U ovom istraživanju analizirana je cvekla iz organskog sistema proizvodnje koja potiče od 6 sertifikovanih proizvođača organske hrane sa različitih geografskih lokacija iz Srbije. Analizirani su sveži uzorci, pasterizovan sok (tretirani na temperature 70 ^{o}C i 90 ^{o}C) kao i sušeni uzorci organske cvekle (na 55 ^{o}C). Utvrđeno je da na koncentraciju vitamina C, sadržaj ukupnih fenolnih jedinjenja (TPC) i antioksidativnu aktivnost (TAA) u cvekli utiče geografska lokacija proizvođača i način prerade. Najveća degradacija za sve analizirane parametre izmerena je u sušenim uzorcima, zatim pri pasterizaciji soka na 90 ^{o}C. Najmanji gubici ispitivanih fitohemijskih komponenti bili su pri pasterizacija soka na 70 ^{o}C. Pokazana je visokoznačajna korelacija između TPC i TAA.

Ključne reči: *pasterizacija, sok, sušenje, fitohemikalije, degradacija, geografsko poreklo*

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