Bulgarian Chemical Communications, Volume 51, Issue 3 (pp.400-405) 2019 DOI: 10.34049/bcc.51.3.5088

Effect of tomato juice storage on vitamin C and phenolic compounds and their stability over one-year period

N. V. Pavlović[1], J. D. Mladenović[2], N. M. Zdravković[3], Đ. Z. Moravčević[4], D. Ž. Poštić[5], J. M. Zdravković[1*]

[1]*Institute for Vegetable Crops, 11420 Smed. Palanka, Karadjordjeva 71, Serbia*
[2]*University of Kragujevac, Agronomski Fakultet, 32000 Čačak, Cara Dušana 34, Serbia*
[3] *Institute of Veterinary Medicine of Serbia, Vojvode Toze 14, 11000 Belgrade, Serbia*
[4]*University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Beograd-Zemun, Serbia*
[5]*Institute for Plant Protection and Environment, T. Drajzera 9, 11000 Belgrade, Serbia*

Received February 20, 2019; Accepted February 26, 2019

The stability of bioactive components: vitamin C and phenols in thermally processed tomato juice for 12 months in three different storage conditions was studied. The experiment was carried out on thermally treated tomato juice made from a selected line of industrial tomatoes (SPO-109). Mashed tomato juice without skin and seeds was cooked for 7 min at 100ºC. The samples were stored in the light at +20ºC; in the dark at +20ºC; and in the dark at +4ºC. The measurement of the changes of the bioactive components parameters during 12 months of storage was performed at 2 month-intervals. Degradation of phenols was not significant for 12 months of storage. Different ambient conditions caused significant changes and loss of nutrients. Vitamin C was lost when stored in the light for the first two months of storage (30.35%), and after one year from 41.56% for storage in the dark at +20ºC (24.58%), and 25.51% at +4ºC. There was no difference in vitamin C content when stored in dark conditions at different temperatures, while significantly important differences were found between storing in the light and storing in the dark at + 20ºC and + 4ºC, respectively.

Key words: storage, different conditions, tomato juice, vitamin C, phenols

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is a vegetable that is consumed fresh (in a salad) and also processed: dried, in sauces, juices, etc. Industrial processing usually includes thermal treatment and/or homogenization and both ways disrupt the cell matrix of tomato fruit [1]. Disruption of the cell wall structure during thermal processing directly affects the availability of different nutrients. Tomato fruits are a major part of human diet since they are an important source of substances with positive effects on health with its vitamins, minerals and antioxidants [2]. Tomato is a plant with antioxidative impact against wide spectra of biotic and abiotic stresses people are exposed to [3]. Furthermore, processed fruits when stored in an adequate way can represent a significant source of bioactive nutrients.

The level of ascorbic acid (vitamin C) in tomato fruit varies from 10 to 88 mg/100 g of fresh weight. Ascorbic acid has an important role in human diet and its intake prevents different diseases [4]. This range of variation appears as a consequence of diversified tomato breeds, and differences among cultivation procedures [5]. Vitamin C belongs to a group of thermally labile and its degradation starts at 70ºC. Significant losses of ascorbic acid can appear in fruits after picking, during storage, however the highest losses appear during processing, when exposed to high temperatures. This degradation appears as a consequence of oxidation and impact of high temperatures [6]. Cold treatment i.e. low temperatures better preserve vitamin C during processing than high temperatures [7]. Besides the climatic conditions the content of vitamin C depends on genotype impact, crop management, fertilizers, fruit development, maturation, and senescence [8,9], which interacting with one another give more or less quality raw material (tomato fruit) used for processing. Maintenance of vitamin C in processed products mainly depends on the length of storage time. In long-term storage conditions, maintenance depends primarily on the influence of climate factors in the warehouse [10]. Increasing the nutritive quality of processed tomato fruit by increasing / preserving the high content of ascorbic acid is always a desirable goal [11].

There are only a few studies regarding the research of phenol preservation in finished tomato products during storage longer than 6 months. Additional research is needed to determine the nutritional quality and its changes during storage, with an emphasis on the phenol changes in ketchup and tomato juice [12]. The storage of tomato juice

* To whom all correspondence should be sent:
E-mail: jasna.zdravkovic@gmail.com

and ketchup results in a mild decrease of polyphenols over time. Degradation of phenolic compounds during storage is attributed to the residual peroxidase activity involved in the oxidative degradation of phenolic compounds. Ketchup had greater stability during storage of tomato juices after 9 months at +4ºC [13]. However, Pérez-Vicente *et al.* [14] found slight changes in the content of total phenolic compounds in juices stored less than 6 months at room temperature. Maintenance of total phenolic compounds during storage could be due to the inactivation of the enzymes responsible for their degradation. Degradation of phenolic compounds during storage was determined at +40°C and higher [15]. Storage time has a significant effect on the preservation of the phenol content and antioxidant activity [16]. Temperature conditions and length of storage are the basic factors of preservation of phenolic compounds in products from thermally processed tomatoes.

This experiment aimed to analyze and determine the level of change of certain bioactive components (vitamin C and total phenols) during long storage (12 months) in thermally processed tomato juice. During the long storage the experimental groups were exposed to different temperatures in light and in dark (which realistically reflects the conditions of storage of tomato juices in markets) in order to find the optimal conditions and length of storage that could benefit human health.

MATERIALS AND METHODS

The experiment was conducted on thermally processed tomato juice of a selected line of industrial tomatoes (SPO). Juice from mashed tomatoes without skin and seeds was cooked for 7 min at 100ºC. Hot juice was poured in glass bottles and hermetically sealed. The samples were stored in glass packaging, in the light at +20ºC; in the dark at +20ºC and in the dark at +4ºC. The measurement of changes of parameters of bioactive components (vitamin C, total phenols and) during the year of storage was performed every 2 months (totally 7 measurements during the experiment: 0-made juice, 2, 4, 6, 8, 10, 12 months storage).

Determination of vitamin C

Pale tomato juice was obtained by pressing 100 cm^3 of tomato juice and mixed with equal quantity (100 cm^3) of a mixture of HPO_3 and glacial CH_3COOH. Then, the mixture was filtered through creased filter paper. The first 5-10 cm^3 of filtered mixture were 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 filtered sample (containing 5 cm^3 of juice and 5 cm^3 of a mixture of HPO_3 and glacial CH_3COOH) was applied to three Erlenmeyer flasks using a pipette. Each sample was titrated with Tillmans reagent (TR) solution until pale pink, for about 5 sec. At the same time, the solution of TR was titrated and blind tested until pale pink [18].

The content of ascorbic acid (mg/cm^3) = $(V - V_1) \times T \times 100/g$

where: V – cm^3 of TR solution used for titration in trial testing; V_1 - cm^3 of TR solution used in blind testing; T– titer solution TR (mg $C_6H_8O_6$ /1 cm^3 TR solution); g – juice volume in cm^3 in the aliquot part of sample.

Total phenols content

Total phenols in the tomato ethanol extracts of 20 g tomato juice, 100 cm^3 in ethanol were estimated according to the Folin–Ciocalteu method [27]. The extract was diluted to a concentration of 1 mg/mL, and aliquots of 0.5 mL were mixed with 2.5 mL of Folin–Ciocalteu reagent (previously 10-fold diluted with distilled water) and 2 mL of $NaHCO_3$ (7.5%). Aliquots were left for 15 min at 45^0C, and then the absorbance was measured at 765 nm on 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 expressed as mg of gallic acid equivalents per gram of dry extract (mg of GA/g).

Data analysis

Genotype differences were determined according to ANOVA model for random block system. Differences between the levels of bioactive components in fresh fruits and products, ratio juice storage: light, 20ºC, dark, 20ºC and dark, 4ºC, were shown according to significant differences by the Tukey's test.

Storage in three ambient conditions was followed by a trend line. This line represents the average state of the observed phenomena through time. Model of linear trend [19]) applied was:

$$\hat{y}=a+ bx,$$

$$\text{where: } b= \sum x_t y_t - n \cdot \dot{x} \cdot \bar{y} / \sum x_t^2 - n \cdot \dot{x}^2,$$

$$a= \bar{y} - \dot{x} \cdot b$$

Multiple correlation and regression represents an extended dependence of one parameter (storage in conditions of light at +20ºC) on impact of several independent variables (storage in the dark at +20ºC and +4°C, respectively) on individual maintenance of bioactive components during long-term storage [20].

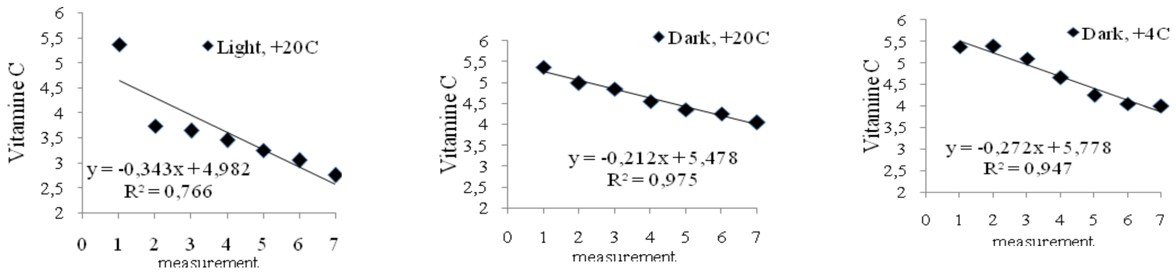
RESULTS AND DISCUSSION

According to the results of storing tomato juice for 12 months in different conditions, the loss of vitamin C was determined. The highest loss in this experiment was at storage in light at +20°C, where the degradation process was the fastest. Trend of vitamin C loss was in linear shape in all followed parameters in the experiment; the determination coefficient at storage at +20°C in light had the lowest value R^2=0.66, while for storage in the dark at +20°C this coefficient was R^2=0.9759 and R^2= 0.974 at +4°C, which confirms the linear shape of vitamin C loss (Fig. 1).

There were significant differences between storage conditions in the dark at both temperatures (+20°C and +4°C) and storage in light, proven by Tukey's test, with 95% accuracy. There were no significant differences among storage in the dark at both temperatures (Table 1). Similar results can be confirmed based on correlation coefficients. All three storage conditions showed high correlation between each other (Table 2).

Percentage of loss of vitamin C in the first two months was 30.35% and after one year 48.79%, when stored at light at +20°C. However, when stored in the dark the situation was clearly different: in the first two months the loss was 7.08% (at +20°C), and when stored in a chilled room at +4°C there were no losses during this period (Table 3).

There were no statistically significant differences in vitamin C content between tomato juice containers kept in dark (20°C and 4°C) while significant differences were found among containers stored in the light and in the dark (20°C and 4°C, respectively), Table 1.



A B C

Figure 1. The average values and tendency of vitamin C depletion during storage for 12 months in different conditions (A- light +20°C, B- dark +20°C, C- dark +4°C).

Table 1. Tukey's multiple comparison test for differences in vitamin C content during storage time in different conditions

Tukey's Multiple Comparison Test	Mean difference	q	Summary	p=95%
Light, +20C : Dark, +20°C	-1.023	4.107	*	-1.922 to -0.124
Light, +20C : Dark, +4°C	-1.080	4.336	*	-1.979 to -0.181
Dark, +20°C : Dark, +4°C	-0.057	0.229	ns	-0.956 to 0.842

*p≤0.05; ns not significant

Table 2. Partial correlation coefficient of vitamin C content in different storage conditions

	Dark, +20°C	Dark, +4°C
Light, +20°C	0.923**	0.783 **
Dark, +20°C		0.958**
Dark, +4°C		

*p≤0.05: **p≤0.01; ns not significant

Table 3. Percentage of vitamin C depletion during 12 months of storage of pasteurized tomato juice

Storage time (months)	Light, +20°C	% loss	Dark, +20°C	% loss	Dark, +4°C	% loss
0m	5.37		5.37		5.37	
2m	3.74	30.35	4.99	7.08	5.37	0.00
4m	3.64	32.22	4.85	9.68	5.1	5.03
6m	3.45	35.75	4.55	15.27	4.65	13.41
8m	3.25	39.48	4.35	18.99	4.25	20.86
10m	3.05	43.20	4.25	20.86	4.05	24.58
12m	2.75	48.79	4.05	24.58	4.00	25.51

Temperature and type of processing affect the vitamin C loss during storage. During thermal treatment vitamin C is destroyed usually by reactions of oxidation at high temperatures in presence of air [21]. The losses of vitamin C were higher than 50% in juices pasteurized at high temperatures during 21 days of storage, and there was 50% decrease of vitamin C in the tomato juice sample in the first 30 days of storage [22]. Significant decrease of vitamin C during the first month of storage was due to oxygen presence in the package and it is probably responsible for most of the loss during long-time storage, according to Min and Zhang [22].

Odriozola-Serrano *et al.* [17] found significantly higher level of vitamin C in tomato juice obtained by pasteurization at low temperatures compared to high temperatures. In their research they proved that during the first week of storage the concentration of vitamin C was higher in tomato juice treated by light pasteurization. They also found no significant difference in vitamin C content between the 7th and 21st day of storage, although this time of storage is considered to be short, which cannot be projected at maximal length of tomato juice storage (one year). The level of phenols in tomato juice during 12 months varied depending on storage conditions (Fig. 2). When stored in the light at +20°C losses were slow, while after 10 and 12 months the loss of phenols in tomato juice was fast (Fig. 2 A), unlike vitamin C which was lost the most in the first months of storage and later had a slow trend (Fig. 1 A).

Losses in phenol content during storage in the dark on both experimental temperatures had similar dynamic and line tendency, which was proved by the high values of determination coefficient (R^2) close to 1 (Fig. 1 B and C). Loss of phenol content at light at +20°C was 80.49% while in dark it was 33.90 and 29.79%, in dark at +20°C and +4°C respectively (Table 4). It is interesting that the level of phenols did not differ in dynamics of loss in the first eight months of storage regardless of different experimental conditions. A critical point is particularly noticeable, where the percentage of loss after 8 months was 25.62%, while after 10 months it increased to 77.14% when stored in the light at + 20°C (Table 4). There were no statistically significant differences of loss of phenol at different storage conditions, as shown in Table 5.

Generally, thermal treatment of tomato fruits causes loss of total phenol content [23]. Thermally treated tomato products displayed a significant loss of phenol components (chlorogenic acid) from 4.4 to 3.5- 3.8 mg/100 ml FV after 56 days at 4°C [16].

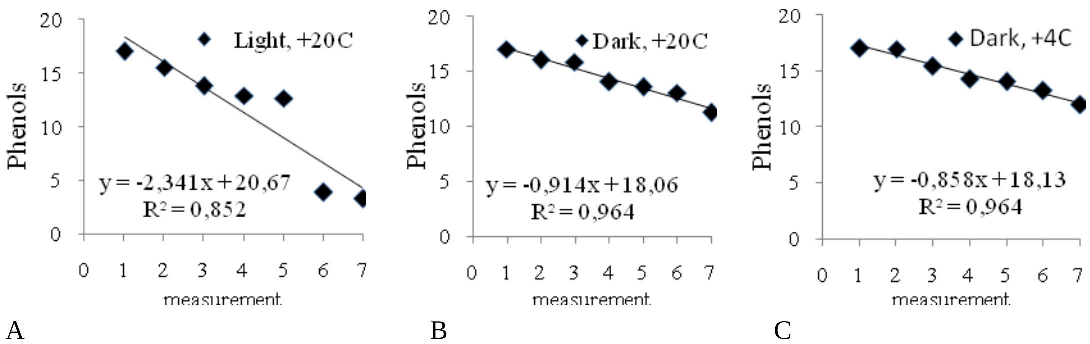


Figure 2. The average value and tendency of phenols loss during 12 months of storage in different conditions A) light +20°C, B) dark +20°C, C) dark +4°C.

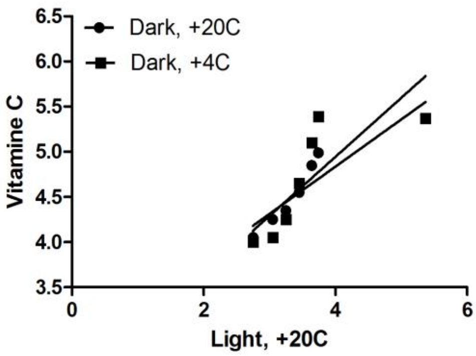
Table 4. Percentage of phenol loss in pasteurized tomato juice during 12 months of storage

Storage time (months)	Light, +20°C	% loss	Dark, +20°C	% loss	Dark, +4°C	% loss
0m	17.02		17.02		17.02	
2m	15.55	8.64	16.05	5.70	16.98	0.24
4m	13.80	18.92	15.85	6.87	15.41	9.46
6m	12.89	24.27	14.05	17.45	14.25	16.27
8m	12.66	25.62	13.55	20.39	14.05	17.45
10m	3.89	77.14	13.05	23.33	13.25	22.15
12m	3.32	80.49	11.25	33.90	11.95	29.79

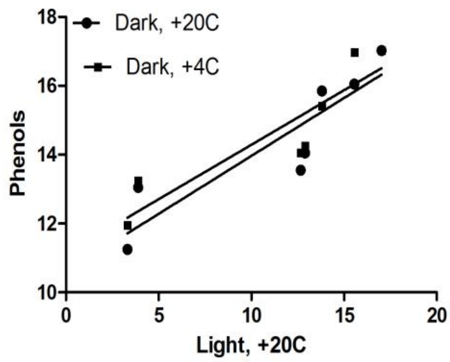
Table 5. Tukey's multiple comparison test of differences in phenols content during storage time at different conditions

Tukey's multiple comparison test	Mean difference	q	Summary	95%
Light 20°C : Dark, +20°C	-3.099	2.315	ns	-7.928 to 1.731
Light 20°C : Dark, +4°C	-3.397	2.544	ns	-8.227 to 1.433
Dark, 20°C : Dark, +4°C	-0.299	0.224	ns	-5.128 to 4.531

ns not significant



A) $x=0.143+0.844x^2-0.09\ x^3$
$p=0.003^{**}$, $p=0.0372^{**}$



B) $x=1.369+2.695x^2-1.953x^3$
$p=0.0067^{**}$, $p=0.0058^{**}$

Figure 3. Regression dependence of tomato juice storage at +20°C and storage in the dark at +20°C and +4°C, respectively.

The highest significant loss of phenols was found by Klimczak *et al*. [24] in storage from 0-6 months, while the loss of phenol was stable and in linear tendency when stored for 4 months at +4 and +20°C [25,26]. Parallel analysis of polyphenols degradation and decrease of hydrophile antioxidative capacity proved the significant loss of polyphenols during storage from 0-6 months (7-12%), while the antioxidative capacity dropped after 9 months of storage (10-12%) [12]. Although, differently from these studies of phenol content during the storage period, Pérez-Vicente *et al*. [14] found slight changes in total phenolic compounds in tomato juices stored for 4 months at + 18 ° C.

The analysis of the regression dependence between the investigated methods of storage (storage in light as a dependent variable compared to independent variables related to storage in dark conditions and at different temperatures), and partial regression coefficient, proved the statistical significance of storage conditions in which tomato juice was kept (Fig. 3 A, B). Regardless to comparison tests performed for the whole storage stream (Tukey's Multiple Comparison Test) partial coefficients of regression determined more specifically the changes after 12 months of storage in function of dynamics of loss of certain bioactive nutrients. This analysis of degradation during storage explains that the best way of storage was storage in the dark. The significance was in dynamics of degradation which was in function of way storage of tomato juice (Fig. 3 A, B). Degradation according to a simple comparison test (Tukey's test) for phenol content was not significant for long-time storage for 12 months.

However, ambient conditions caused a significant loss of these components, especially when stored in light, compared to storage in the dark (partial coefficient of multiple regression, Fig. 3) since dynamics of degradation in the first months was slower.

CONCLUSION

The increase in people's awareness of the need to nutrient value with added value as health improvement substance has put tomato and its products on an important position in human diet. Tomato juice is its very popular product, consumed quite a lot.

This experiment proved that degradation of total phenols from tomato juice during 12 months of storage was not significant. Also, the experiment proved that the conditions of storage of tomato juice significantly impact on its nutritive compounds. Degradation was caused by storage at light compared to dark. Total content of vitamin C in processed tomato juice was found after the first two months of storage at light (30.35%). The obtained experimental data could be very useful for choice of adequate warehouse for long-term storage of tomato juice in the perspective of preserving vitamin C and total phenols as health-valuable nutrients.

Acknowledgement: *This study was supported by the Serbian Ministry of Education, Science and Technological Development: Projects No. TR31059 (Integrating biotechnology approach in breeding vegetable crops for sustainable agricultural systems).*

REFERENCES:

1. K. H. Van het Hof, B.C. De Boer, L. B. Tijburg, B. R. Lucius, I. Zijp, C. E. West, J. G. Hautvast, J.A. Weststrate, *J. Nutr.*, **130**, 1189 (2000).
2. L. Frusciante, P. Carli, M. R. Ercolano, *Mol. Nutr. Food* Res., **51**, 609 (2007).
3. R. D. Gallie, *Scientifica*, **1,** 24 (2013).
4. A. K. Schlueter, C. S. Johnston, *J. Evid. Based Complement. Altern. Med.*, **16**, 49 (2011).
5. V. Locato, S. Cimini, L. D. Gara, *Front. Plant Sci.*, **4**, 152 (2013).
6. B. A. Fox, A. G. Cameron, Food science, nutrition & health, 6th edn., (Chapter 13: Vitamins). J.W. Arrowsmith Ltd., Bristol, UK, 1995, p. 236.
7. W. Davey, D. Van Montaguinze, M. Sanmartin, A. Kanellis, N. Smirnoff, J. Benzie, J. Strain, D. Favell, J. Fletcher, *J. Sci. Food Agr*., **80**, 825 (2000).
8. R. K. Toor, G. B. Savage, A. Heeb, *J. Food Comp. Anal*. **19**, 20 (2006).
9. G. R. Borguini, D. H. Markowicz, J. M. Moita-Neto, F. S. Capasso, E. A. Torres, *Braz. Arch. Biol. Technol.*, **56**, 521 (2013).
10. A. Raiola, M. M. Rigano, R. Calafiore, L. Frusciante, A. Barone, Mediators of Inflammation, ID 139873, 2014, p. 16.
11. V. Ruggieri, B. B. Hamed Amalia, L. Frusciante, M. L. Chiusano, *Plant Mol. Biol*., **91**, 397 (2016).
12. A. Vallverdu-Queralt, S. Arranz, A. Medina-Remon, I. M. Casals-Ribes, R. Lamuela-Raventos, *J. Agric. Food Chem.*, **59**, 9358 (2011).
13. A. Vallverdu-Queralt, A. Medina-Remón, I. Casals-Ribes, C. Andres-Lacueva, A. L. Waterhouse, R.M. Lamuela-Raventos, *LWT - Food Sci. Tech*., **47**, 154 (2012).
14. A. Pérez-Vicente, P. Serrano, P. Abellán, C. García-Viguera, *J. Sci. Food Agr*., **84** (7), 639 (2004).
15. V. Lavelli, G. Giovanelli, G., *J. Sci. Food Agr.,* **83**, 966 (2003).
16. I. Odriozola-Serrano, R. Soliva-Fortuny, V. Gimeno-Ano, O. Martin-Belloso, *J. Food Eng.,* **89**, 210 (2008).
17. I. Odriozola-Serrano, R. Soliva-Fortuny, O. Martín-Belloso, *Food Sci. Emerg. Technol*., **9**, 272 (2008a).
18. Praktikum iz Biohemije, M. Cvijović, G. Aćamović-Đoković (eds.), Agronomski Fakultet, Čačak (srb), 2005, p. 250.
19. Osnovi statistićke analize, R. Njegić, M. Žižić, M. Lovrić, D. Pavličić (eds.), Savremena administracija, Beograd, 1991, p. 498.
20. R. A. Hoshmand, Statistical methods for environmental & agricultural sciences, 2nd edn., CRC Press, Boca Raton, New York, USA, 1998, p. 439.
21. C. Leoni, Improving the nutritional quality of processed fruits and vegetables: the case of tomatoes, in fruit and vegetable processing: Improving quality. W. Jongen (ed.), Woodhead Publishing Ltd and CRC Press, LLC, Cambridge, 2002, p. 83.
22. S. Min, Q. H. Zhang, *Food Chem. Toxicol*., **68**, 1600 (2003).
23. R. Pavlović, J. Mladenović, N. Pavlović, N., M. Zdravković, D. Jošić, J. Zdravković, *Acta Sci. Pol. Hort. Cult.*, **16**(3), 119 (2017).
24. I. Klimczak, M. Malecka, M. Szlachta, A. Gliszczynska-Swiglo, *J. Food Compos. Anal*., **20**, 313 (2007).
25. G. Giovanelli, A. Paradiso, Stability of dried and intermediate moisture tomato pulp during storage. *J. Agric. Food Chem*., **50**, 7277 (2002).
26. A. Gliszczynska-Swiglo, B. Tyrakowska, *J. Food Sci.*, **68**, 1844 (2003).
27. V. L. Singleton, R. Orthofer, R. M. Lamuela-Raventós, *Methods Enzymol*., **299**, 152 (1999).