Bulgarian Chemical Communications, Volume 51, Issue 4 (pp. 604 - 610) 2019 DOI: 10.34049/bcc.51.4.5126

Effects of tomato processing on carotenoids antioxidant activity and stability during one-year storage

J. M. Zdravković[1*], N. V. Pavlović[1], J. D. Mladenović[2], N. M. Vragolović Bošković[3], N. M. Zdravković[4]

[1] *University of Kragujevac, Faculty of Agriculture, Cara Dušana 34, Čačak, Serbia*
[2] *Institute for Vegetable Crops, Karadjordjeva 71, 11420 Smederevska Palanka, Serbia*
[3] *University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, Belgrade, Serbia*
[4] *Institute of Veterinary Medicine of Serbia, Vojvode Toze 14, Belgrade, Serbia*

Received April 1, 2019; Accepted May 28, 2019

The degradation of carotenoids (lycopene and *β*-carotene) and total antioxidant activity was investigated after one-year storage of pasteurized tomato juice. Tomato juice, thermally treated for 7 min at 100°C, was subjected to one-year storage a) in the light at 20°C; b) in the dark at 20°C and c) in the dark at 4°C. *β*-Carotene had the fastest dynamics of degradation and was dissolved in the largest quantities, regardless of the storage conditions. For all investigated components the fastest decomposition was observed in the first two months, when the sample was stored in the light at 20°C. Lycopene was most stable in the sample stored in the dark at 4^{0}C. Partial regression coefficients for all researched traits proved a significant difference of ratio for storing in the light (20^{o}C) compared to the variants stored in the dark at 20^{o}C and 4^{o}C, lycopene p=0.0041**, p=0.0304**; *β*-carotene, p=0.0009** and p=0.0183**; antioxidative activity p<0.0001** and p=0.009**.

Keywords: tomato juice, 1-year storage, lycopene, *β*-carotene, antioxidative activity

INTRODUCTION

In the last two decades consumption of tomato in the world has significantly increased, mostly due to its nutritional importance and beneficial effects on cardiovascular diseases [1-3] and some cancers. Demand is focused on products based on tomato (ketchup and tomato juice) which, like fresh tomatoes, have antioxidant, anti-inflammatory, anti-mutagenic and anti-cancerous impact [4,5]. Tomato fruits can obtain optimal combination of antioxidants, so they are therefore important for human diet. When it comes to storing and processing of tomato fruits, research studies have examined most particularly the loss of nutrients, changes that occur during exposure to high temperatures, the length of processing time, the influence of light or oxygen, the length of shelf life [6,7]. Only few researchers studied the problem of storing products and decomposition process of some nutrients and length of storage [8]. However, technological processing and storing conditions influence the nutritive quality of tomato products and their stability to varying degrees [9]. Of all the processing techniques, high temperatures have the greatest impact on the level of natural nutrients in vegetables. Depending on the length of treatment, the level of impact can be high or low [10]. Beta-carotene and lycopene participate with 7 and 87%, respectively, of total carotenoids in mature red, tomato fruits [11].

Lycopene belongs to a family of carotenoids in its natural all-*trans* form. In tomato juice, as a result of oxidation, lycopene molecules divide, which leads to discoloration and bad taste. Effects of heat, oxygen, light and presence of oils on the lycopene stability have been proved in many studies. Opposing data regarding stability of tomato carotenoids during thermal treatment can be found in the literature.

Interestingly, while some studies show that compounds with antioxidative effects such as lycopene or *β*-carotene during some processing could be increased comparing to fresh fruit [12,13], other results show that the lycopene levels do not significantly vary in tomato puree packages if stored in the dark at 5, 15 and 25°C and in the light at 25°C for 6 months [14]. Also Tamburini *et al*. [15] did not find any changes in the lycopene content of tomato puree during storing for 1 year time, an increase in the lycopene level and stable *β*-carotene in processed tomato product have been published [7, 16]. After industrial processing *β*-carotene and lycopene remained stable during 12 months of storage depending on the terms of storage [17].

However, there are some controversies about some circumstances that lead to occurrence of isomerisation, such as optimal humidity and storage temperature [18]. Processed tomato products such

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

© 2019 Bulgarian Academy of Sciences, Union of Chemists in Bulgaria

as pulp, puree and paste exhibit degradation of antioxidants and antioxidative activity after 3 months of storage in each temperature conditions (30, 40 and 50°C) [19]. As the time of the storage increases for all storage treatments, the level of lycopene significantly decreases [20,21]. There is also a problem with the change in colour during storage. Even without oxygen to start the oxidation process, lycopene is slowly decomposed by auto-catalytic mechanism [22]. There are few research data on the level of lycopene after thermal treatment of stored tomato products in final food preparation procedures, i.e the amount of lycopene that consumers actually intake [23]).This appoints to attention for *cis*-lycopen supply in order to optimise tomato processing and storage condition in order to provide high quality food rich in nutrients and additional antioxidant value.

The other flavonoid in this research focus, *β*-carotene, is 20% lost after 7 months of storage [24], also after as much as 3 months of storage at 30, 40 and 50°C, the level of *β*-carotene decreased [19]. Similar result was presented in the study [25], where the loss of *β*-carotene and lycopene has been compared to the loss of ascorbic acid (vitamin C). Carotenoids were lost during storage but significantly less comparing to the thermo-labile ascorbic acid, which was significantly lost during heat treatment (compared to fresh fruits) and this loss is even greater during storage.

Antioxidative capacity is connected to the quantity and composition of bioactive compounds from the natural combination of different phyto-nutrients [26,27]. A certain number of studies proved that processed vegetables have the same nutritive value as fresh. This directly impacts the end users regarding processed food and increases the conscience of the health benefits of the processed vegetables in prevention of chronic diseases. Dewanto *et al*. [28] and Perez-Conesa *et al*. [29] proved that the high level of anti-oxidative capacity of products was preserved despite the heat treatment and loss of a strong antioxidant such as ascorbic acid. Total antioxidative activity was significantly increased after heat treatment for 2, 15 and 30 min at 88°C to 5.29 $\pm$0.26, 5.53 $\pm$0.24 and 6.70 $\pm$0.25 μmol during the processing. Similar results had Odriozola-Serrano *et al*. [30] who found significant changes of antioxidative capacity among treated and fresh juices right after processing. However, after 56 days of storage at 4ºC the antioxidative activity dropped to 35.7%, and authors concluded that antioxidative activity may suffer significant reduction in tomato products towards expiration date [19].

Different behaviour of carotenoids and total antioxidative activity in heat-treated mature tomato fruits juice should result in one-year research of expiration date which would contribute to clarification of degradation of bioactive components during storage time in preserving tomato products as high nutritive level food.

MATERIAL AND METHOD

Juice was prepared from one genotype – selected, high inbreeded tomato line (SPO).

Epidermis and seeds were separated. Pulp was thermally treated (cooked) at 100ºC for 7 minutes and aseptically poured in glass containers which were hermetically sealed by metal lid. Samples were stored for one year in three conditions: at day light at 20ºC, in the dark at 20ºC and in the dark at 4ºC. The lycopene level, *β*-carotene and total antioxidative activity were checked every two months. Testing was done on a sample of ten bottles from each repetition treatment.

Total antioxidative activity (TAA) was determined spectrophotometrically at 517 nm DPPH (1.1-diphenyl-2-pikrylhydrazyl) by applying method [31].

In order to determine the level of lycopene, 20 g of tomato was extracted in 100 cm^3 96% C_2H_5OH. After 24 h of extraction (maceration), the sample was filtered. The dry extract was dissolved in 10 cm^3 of acetone-hexane (ratio 4:6) mixture and filtered through Whatman No.4 filter paper. The extract was diluted 10 times and the absorbance at wavelengths 453, 505, 645 and 663 nm was measured [32]. Spectrophotometric measurements of samples were performed on the UV-VIS spectrophotometer MA9523-SPEKOL 211 (Iskra, Horjul, Slovenia). The level of lycopene (mg lycopene/ 100 mL extract) was calculated:

$$\text{Lycopene (mg/100 mL)} = -0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453}$$

Levels of *β*-carotene were determined according to the method described in [32]. The dried ethanol extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Content of *β*-carotene was calculated according to the following equation:

$$\beta\text{-carotene (mg/100 ml)} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$$

Tendency of change of average values [33], specifically linear-shaped tendency was tested:

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

where:

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

$y = a * x + b$

and logarithmic tendency:

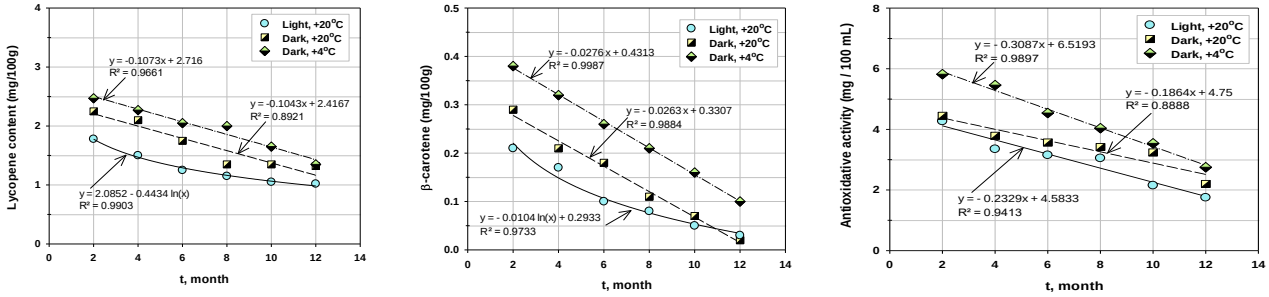
$y = a * \ln(x) + b$,

where: a and b are constants, ln is the natural logarithm.

Adjustment to linear shape tendency was tested by the determination coefficient $R^2 > 0.6$.

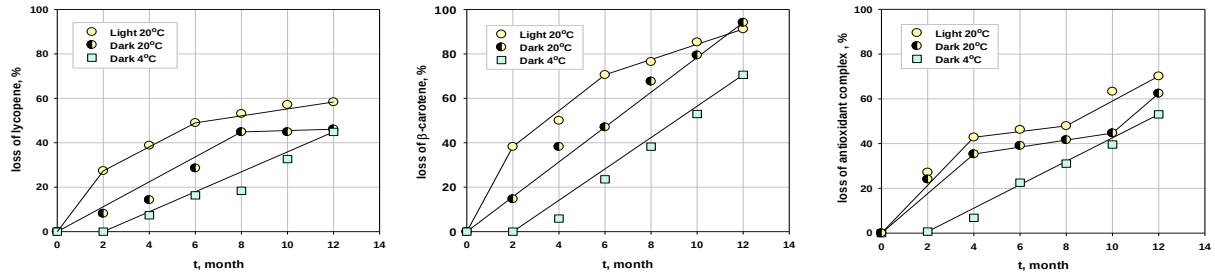
The differences between the way of storage of samples and respective mutual dependence [34] were determined on the basis of partial regression coefficients.

Degradation index for studied nutrients was expressed as the ratio of their respective initial value and values measured in time.



a) lycopene b) *β*-carotene c) antioxidative activity

Figure 1. Average values, tendency to change the content of lycopene (a), *β*-carotene (b) and total antioxidative activity (c), after one-year storage in the light (20°C), in the dark (20°C) and in the dark (4°C)



a) b) c)

Figure 2. Percentage loss of lycopene (a), *β*-carotene (b) and TA activity (c) depending on time and storage conditions.

RESULTS AND DISCUSSION

In the aspect of lycopene levels, the least satisfactory storage conditions are at 20°C in daylight. Tendency of lycopene loss has logarithmic nature for all tree evaluated conditions of tomato juice storage. There is high reliability in predicting lycopene levels as determination coefficients are $R^2 = 0.9903$ for daylight storage conditions, $R^2 = 0.8921$ for dark place storage at 20°C, and $R^2 = 0.9661$ in dark on 4°C (Fig 1a).

Complementary to lycopene, the change in *β*-carotene levels depends on shelf life, and temperature situation of tomato juice storage. Amount of *β*-carotene linearly drops after a whole year of testing in dark at both 4°C and 20°C (both with R^2 values close to 1, 0.9987 and 0.9984 respectively), but in daylight the loss is presented in logarithmic style (Fig. 1b).The TA activity linearly drops for all 3 tested tomato juice storage conditions, with high R^2 values (Fig 1c).

The dependences of lycopene, β-carotene and TA activity loss are presented on Figures 2a-2c, respectively, as a function of storage time. The figures show also the lines that follow the trend of the displayed values and which slope is equivalent to the degradation rate of the corresponding component. The greatest losses of lycopene (up to 58%) were registered in samples that were stored at 20°C in daylight. The rate of lycopene degradation was the highest in the first two months of storage and decreased from the second to the twelfth month.

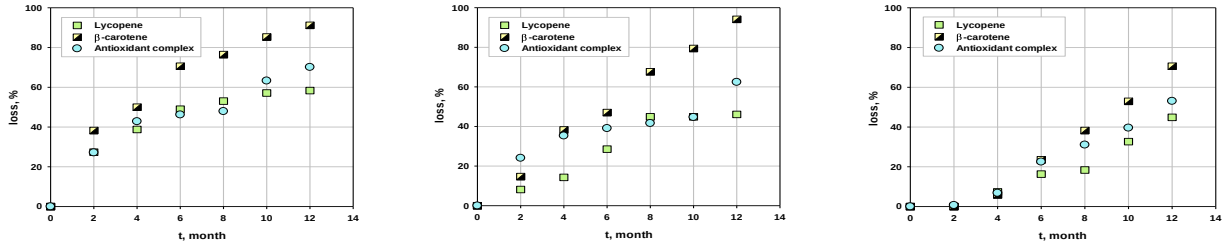
In dark storage at 20°C the rate of lycopene degradation was constant till the 8th month and after that the sample had the same quantity of lycopene, which means that the degradation ends (Fig 2a). The best result for lycopene preservation in tomato juice was for storage in the dark at 4°C. It is

interesting that after 12 months of storage the amount of lycopene in samples stored in the dark at different temperatures was the same (Fig 2a). Hsu *et al*. [35] also found that nutrients degraded most quickly in the first phases of storage. These researchers found that in the first 14 days of storage, carotenoid and lycopene degraded quickly (16% and 12%, respectively) compared to control, while later degradation during 14 to 28 days of storage was not significant. The sample researched in this paper, which was stored in the dark at 20°C had almost the same amount of lycopene since the 8th month of storage which means that there was no degradation of lycopene in the last four months. This is in accordance with Ordonez-Santos *et al*. [20], where researchers aimed to follow the dynamics during storage of tomato pulp up to 180 days (half of the time compared to the storage time in our experiment) where the level of lycopene showed significant changes during the storage period. In some other studies, there were no changes in the level of lycopene in tomato puree during one year of storage [15], while some authors reported increased lycopene level in tomato products comparing to fresh tomato: 36% increase in lycopene and stable *β*-carotene [7] and 30% increase in lycopene [16].

Significant loss of *β*-carotene in all storage conditions is shown on Fig 2b. The parameter which significantly impacts the loss of this compound is the temperature. Whether the sample was stored in light or in dark, the temperature of 20°C caused great losses of *β*-carotene. The rate of degradation changed during storage in light, similar to lycopene, degradation was the most intensive in the first two months, but in time it was less comprehensive. The slowest degradation in daylight at 20 °C, was between the 6th and 12th month of storage. In the first two months there was no drop in *β*-carotene level in tomato juice while stored in cold and dark conditions. In the next 10 months the rate of degradation was constant and same as the rate of degradation in the dark at 20°C.

Lavelli and Giovanelli [19] found that the level of *β*-carotene decreased after 3 months of storage at 30, 40 and 50°C and concluded that storage conditions play an important role in preservation of this particular nitrient during storage.

For preserving TA activity (Fig. 2c), the temperature of storage has fundamental significance. The highest loss was observed in samples stored at 20°C, and less TAA was lost in samples stored at 4°C. During the first four months of storage at 20°C the degradation of TAA was intensive. After the 4th month the rate of degradation of TAA was significantly lower but after the 8th month the rate of degradation of this component was higher. For samples stored in the dark at 4°C, there was no change in TAA during the first two months. After two months of storage, the rate of degradation was constant and all the time the loss of TAA was lower compared to the samples stored at higher temperatures. These conclusions are in accordance with the results obtained by Odriozola-Serrano *et al*. [30], who found a decrease in TAA of 35.7% after 56 days of storage at 4°C, although in our research at the same temperature the level of loss of TAA was lower (Fig. 2c). Three studied parameters lycopene, *β*-carotene and TA activity, as well as the dependence of their losses as a function of storage period in different conditions are shown in Figs. 3a-3c. In all cases, the greatest loss was observed for *β*-carotene, and the smallest one for lycopene. Degradation of carotenoids and especially of *β*-carotene has been explained by Boon *et al*. [36] claiming that carotenoids belong to a group of non-stable bionutrients, with many mechanisms of degradation in some tomato products. Our results are significantly different from those of Koh *et al*. [17], who displayed a stable level of *β*-carotene and lycopene in tomato juice: after one year of observation, the level of lycopene was 96% and *β*-carotene was 100% preserved. According to our results the amount of these bioactive substances was not constant in any storing conditions. Our results are in accordance with those of most researchers who found that the phyto-nutrients were lost during storage [19, 24, 25]. They studied the changes of *β*-carotene in juice after 7 months of storage at 22°C for 12 varieties and found a difference in the level of *β*-carotene comparing fresh and processed fruits. The loss of *β*-carotene was 20% during extraction and other 20% was found after 7 months of storage [19]. Rate of lycopene degradation in juice stored in daylight differs from that on storing in dark at 20°C and in dark at 4°C. After one year, degradation rate of lycopene for storing in light was 2.4; for storing in the dark at 20°C the rate was 1.86 and at 4°C the rate was 1.81 (Table 1).

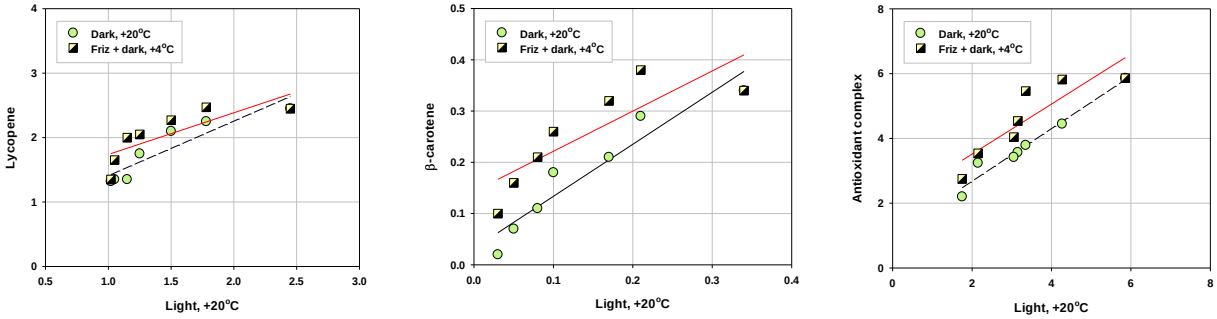


a) light 20 ºC b) dark 20ºC c) dark 4ºC

Figure 3. Percentage of lycopene (a), *β*-carotene (b) and antioxidative activity (c) complex loss depending on time in different storage conditions

Table 1. Index of lycopene and *β*-carotene and antioxidative activity degradation in different conditions during one year

Months of storage	Lycopene			*β*-Carotene			TA activity		
	Light, 20ºC	Dark, 20ºC	Dark 4ºC	Light, 20ºC	Dark 20ºC	Dark 4ºC	Light, 20ºC	Dark, 20ºC	Dark 4ºC
2	1.38	1.09	0.99	1.62	1.17	1.00	1.37	1.32	1.00
4	1.63	1.17	1.08	2.00	1.62	1.06	1.75	1.55	1.07
6	1.96	1.40	1.20	3.40	1.89	1.31	1.86	1.64	1.29
8	2.13	1.81	1.23	4.25	3.09	1.62	1.92	1.71	1.45
10	2.33	1.81	1.48	6.80	4.86	2.13	2.73	1.81	1.66
12	2.40	1.86	1.81	8.50	6.80	3.40	3.35	2.66	2.13



a) $\bar{y}=0.069+1.068x^2-0.272x^3$, p=0.0041**, p=0.0304**

b) $\bar{y}=-0.019+1.067x^2-0.144x^3$, p=0.0009**, p= 0.0183**

c) $\bar{y}=-0.011+0.826x^2+0.133x^3$ p<0.0001**, p=0.009**

Figure 4. Regression analysis of the change in the level of lycopene (a), *β*-carotene (b) and antioxidative activity (c) for storing in light (+20ºC) and in dark (+20ºC and +4ºC)

Our results were partially in accordance with Sharma and Le Maguer [37] who found 2.7 and 7.5 times higher rates of lycopene degradation in thermally treated tomato puree stored for 60 days in the dark at 25ºC comparing to samples stored at 5 and 20ºC, respectively, in the same conditions. Our results were in accordance with those of Min *et al*. [38] who found no significant differences in the level of lycopene for 1.5 months of storage in the dark at 4ºC. In these storing conditions and in this period no degradation was observed in our research. Our results were opposite to those of Rajchl *et al*. [39] who found a decrease in lycopene of 24% in commercial ketchup after 19 days of storage at 25ºC, having in mind that ketchup is a thermally treated tomato juice. The results of this research could be compared with the similar research of Marković *et al*. [14] where tomato juice was stored in the dark at 5, 15 and 25°C and in light at 25°C for 6 months and where the variation of lycopene was not significant comparing to the level of lycopene at the beginning of the storage for a short period of time. With the increase in time of storage for all treatments the level of lycopene significantly decreased. The obtained results of storing in light at 20ºC showed that lycopene quickly degraded comparing to the treatments in dark after 2 months (27.35%), (Fig 1a).

Losses of *β*-carotene in light were 8.50 times higher comparing to one year of storage in light at 20°C. High loss was registered while storing in the dark at 20ºC after 12 months (index of degradation

6.80).When stored in the dark at 4ºC, the losses of β-carotene were the lowest (3.4 times after 12 months) (Table 1). This is in accordance with Gupta *et al*. [21] where storage of samples at 4, 25 and 37^{0}C for 52 weeks resulted in degradation of lycopene and the variation was explained as a function of tomato variety, processing method, temperature and duration of storage.

The regression coefficients pointed to a significant difference between lycopene, β-carotene and TA activity in terms of storage in light and in dark (Fig 3). For all the researched traits, partial regression coefficients proved a significant difference between storage in light (20ºC) and in dark at 20ºC and 4ºC, as well as high dependence of storing in the dark at 4^{0}C. Differences in loss of lycopene, β-carotene and total antioxidative activity were at the level of statistical significance among storing in light (20ºC) and storing in dark both at 20^{0}C and at 4^{0}C. Partial regression coefficients proved high dependence and significant difference in the preservation of antioxidative activity for storing in light in favour of storing at the dark (20ºC and 4ºC) (Fig. 4).

Dynamics of loss was different depending on storing conditions. Repeated thermal treatment of tomato puree exhibited the existence of many mechanisms of degradation: possible auto-oxidation and isomerisation of carotene, as well as increased extraction of carotenoids at high temperatures [40]. Additional information could give the possibility of optimization of processing for obtaining products with high nutritional values after extended period of storage [41]. Tomato products in most cases are thermally treated once more during cooking (depending on the meal prepared). For qualitative products we need more information regarding production process, genotype selection, agro technique of crop growing, determination of optimal time of yield, etc. Understanding of mechanisms of biochemical changes that take place in the tissue during processing is of a great importance. This represents important steps that will lead to innovation in food industry.

CONCLUSION

Generally, from these dependencies the following conclusions can be drawn:

- The fastest and most intense degradation was found for β-carotene regardless of the storing conditions;
- All studied components underwent the fastest decomposition in the first two months, when the sample was kept in light at 20ºC;
- Lycopene was most stable in the sample stored in the dark at 4ºC;
- For all studied components in the sample stored in the dark at 4ºC there was no change in composition in the first two months.

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. H. D. Sesso, S. Liu, J. M. Gaziano, J. E. Buring, *J. Nutr.*, **133**, 2336, (2003).
2. A. Osińska, M. Osiński, A. Krasińska, B. Begier-Krasińska, *Kardiochirurgia i Torakochirurgia Polska*, **14**, 133, (2017).
3. K. Yamagata, Chapter 7 Genetics and Molecular Biology, Carotenoids, 2017. DOI: 10.5772/67464.
4. J. K. Willcox, G. L. Catignani, S. Lazarus, *Crit. Rev. Food Sci*., **43**, 1 (2013).
5. S. M. D. Grieb, R. P. Theis, D. Burr, D. Benardot, T. Siddiqui, N. R. Asal, *J. Am. Diet. Assoc*., **109**, 656, (2009).
6. J. Shi, M. Le Maguer, *Crit. Rev. Biotec*., **20**, 293, (2000).
7. A. A. Abushita, H. G. Daood, P. A. Biacs, *J. Agric. Food Chem*., **48**, 2075 (2000).
8. S. M. Wasim, J.P. Singh, *J. Chem. Environ.*, **19**, 82, (2015).
9. F. J. García-Alonso, S. Bravo, J. Casas, D. Pérez-Conesa, K. Jacob, M. L. Periago, *J. Agr. Food Chem.*, **57**, 6815, (2009).
10. D. Murador, D. Cunha, V. Rosso, *Food Res. Int*., **65**, 177, (2014).
11. J. Singh, M. Rai, *Indian J. Hortic*., **54**, 33, (2006).
12. G. Graziani, R. Pernice, S. Lanzuise, P. Vitaglione, M. Anese, V. Fogliano, *Eur. Food Res. Technol*., **216**, 116 (2003).
13. C. H. Chang, H. Y. Lin, C. Y. Chang, Y. C Liu, *J. Food Eng*., **77**, 478, (2006).
14. K. Marković, M. Hruška, N. Vahčić, *Acta Aliment. Hung*., **36**, 89, (2007).
15. R. Tamburini, L.Sandei, A. Aldini, F. De, C. Leoni, *Industria Conserve*, **74**, 341, (1999).
16. R. Re, P. M. Bramley, C. Rice-Evans, *Free Radical Res.*, **36**, (2002).
17. E. Koh, S. Charoenprasent, A. E. Mitchell, *J. Sci. Food Agr.*, **92**, 23, (2012).
18. S.Xianquan, J. Shi, Y. Kakuda, J. Yueming, *J. Med. Food*, **8**, 413, (2005).
19. V. Lavelli, G. Giovanelli, *J. Sci. Food Agr*., **9**, 966 (2003).
20. L. E. Ordonez-Santos, L. Vazquez-Oderiz, E. Arbones-Macineira, M. A. Romero-Rodriguez, *Food Chem*., **112**, 146 (2009).

21. R. Gupta, V. M. Balasubramaniam, S. J. Schwartz, D. M. Francis, *J. Agr. Food Chem*., **58**, 8305 (2010).
22. J. S. Smith, Y. H. Hui, Food Processing, Principles and applications, Blackwell Publishing Company, USA, 2004, Print ISBN: 9780813819426, Online ISBN: 9780470290118.
23. G. B. Martinez-Hernandez, M. Boluda-Aguilar, A. Taboada-Rodriguez, S. Soto-Jover, F. Marin-Iniesta, A. Lopez-Gomez, *Food Eng. Rev*., **8**, 52 (2015).
24. J. M. Dietz, W. A Gould, *J. Food Sci*., **51**, 847 (2006).
25. A. Vashista, A. Kawatra, S. Sehgal, *Plant Food Hum. Nutr*., **58**, 1 (2003).
26. M. V. Eberhardt, C. Y. Lee, R. H. Liu, *Nature*, **405** (6789), 903 (2000).
27. C. Sánchez-Moreno, L. Plaza, B. de Ancos, P. M. Cano, *Food Chem*., **98**, 749 (2006).
28. V. Dewanto, X. Z. Wu, K. K. Adom, R. H. Liu, *J. Agr. Food Chem*., **50**, 3010, (2002).
29. D. Perez-Conesa, J. Garcia-Alonso, V. Garcia-Valverde, M. D. Iniesta, K. Jacob, L. M. Sanchez-Siles, *Innov. Food Sci. Emerg. Technol.*, **10**, 179 (2009).
30. I. Odriozola-Serrano, R. Soliva-Fortuny, V. Gimeno-Ano, O. Martin-Belloso, *J. Food Eng.*, **89**, 210 (2008).
31. F. Xu, L. Li, X. Huang, H. Cheng, Y. Wang, S. Cheng, *J. Med. Plants Res.*, **4**, 2544 (2010)
32. M. Nagata, I. Yamashita, *J. Food Sci. Tec.*, **39**, 925 (1992).
33. R. Njegić, M. Žižić, M. Lovrić, D. Pavličić (Fundamentals of Ancient Analysis - III Edition). Beograd, Srbija: Savremena administracija, 1991.
34. R.A. Hoshmand, Statitical Methods for Environmental et Agricultural Sciences. (2nd edition) CRC Press, Boca Raton, New York, USA. 1998, ISBN: 0-8493-3152-8.
35. K.C. Hsu, F. J. Tan, H. Y. Chi, *LWT – Food Sci. Technol*., **41**, 367 (2008).
36. C.S. Boon, D. Mcclements, J. Weiss, E. A. Decker, *Crit. Rev. Food Sci*., **50**, 515 (2010).
37. S.K. Sharma, M. Le Maguer, *Ital. J. Food Sci.*, **2**, 107, (1996).
38. S. Min, Z. T. Jin, Q. H. Zhang, *J. Agr. Food Chem*., **51**, 3338, (2003).
39. A. Rajchl, M. Voldrich, H. Čižkova, M. Hronova, R. S. Evcik, J. Dobias, J. Pivonka, *J. Food Eng*., **99**, 465 (2010).
40. S. Luterotti, D. Bicanic, K. Marković, M. Franko, *Food Control*, **48**, 67 (2015).
41. E. Capanoglu, J. Beekwilder, D. Boyacioglu, R. C. H. De Vos, R. D. Hall, *Crit. Rev. Food Sci.*, **50**, 919 (2010).