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Following-up the changes in bioactive-antioxidative compounds, as a function of water supply and processing, of industrial tomato cultivated in two different areas using recent analytical methods.

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Introduction and objectives of the research

Tomato (*Solanum lycopersicon* L.) and its products can play a significant role in modern human diet as important sources of vitamins, minerals, and antioxidants as well as their being relatively easily accessible foods. Among phytochemicals, carotenoids are of special interest due to their role in the reduction of the onset of several types of cancer (Giovannucci, 1999) and cardiovascular (Gammone et al. 2015) or neurodegenerative diseases (Rao and Rao, 2007). Tocopherols and vitamin C components play an important role in the defense against oxidative stress (George et al. 2005). The factors most likely to affect the concentration of phytonutrients in the industrial tomato, as in other vegetables may include variety, ripening stage at harvest, agronomical, geographical, and environmental conditions (Lenucci et al. 2006; Ilahy et al. 2011). The nutritive value of tomato fruit can be optimized by the application of suitable cultural practices such as the right choice for topsoil (Meulebroek et al. 2012). In Hungary, industrial tomatoes are grown in different regions with different soil properties (Illés et al., 2016) and may differ in many other environmental parameters. However, many different areas are suitable for the cultivation of industrial tomatoes, but their different agronomic characteristics and abiotic factors may contribute to the different levels of nutritional value of tomatoes and their stability during processing.

The type of technology used in tomato juice processing is another factor that may cause alteration in quality components of the final products. To produce tomato products industrial varieties, which are resistant to mechanical injuries, are to be used. Usually, the cold-break extraction carried out at 50-60°C and hot-break extraction (HBE) carried out at 90 °C are the widely used technological processes to produce tomato juice (Goodman et al. 2002). The extraction of the raw material by heat treatment has several purposes. On the one hand, it allows the inactivation of microorganisms and enzymes, and on the other hand, it helps to break down tissues in order to achieve proper organoleptic properties (Capanoglu et al., 2008).

The main objective of the present work was to investigate how the abiotic factors of the place of cultivation, and the method of processing impact on the content and thermal stability of carotenoids, tocopherols, and vitamin C in industrial tomatoes. To follow the compositional changes on the phytochemicals it is necessary to use the analytical protocol that provides efficient and precise separation and detection of the individual compounds and their isomers and derivatives.

Materials and methods

The experiments were performed at two different locations (Szarvas, Szárítópuszta) with the UG812J (United Genetics Seeds Co., Hollister, CA) industrial tomato hybrid. The two different production areas have several environmental and ecological properties (soils specifications Szárítópuszta: 41% sand, 47.5 sludge and 11.5% clay, Szarvas: 37% clay, 29, 4% sand and 33.6% sludge). The length of the growing season was 100 days both during the 2 years and in the two growing areas. Szárítópuszta is a part of Gödöllő territory, which is characterized by high precipitation and low temperature during the cultivation season of vegetables, in contrast to Szarvas. Szarvas climate is typically drier and warmer than Szárítópuszta.

In the water stress experiment, the irrigation water demand could be used with AquaCorp v5.0 software to calculate the potential evapotranspiration (PET). Subsequently, in both experimental areas, irrigation of one plot with 100% of the calculated PET value (PET_100), i.e., with full replacement of the loss, the other with the calculated PET with 50% (PET_50) or the control plot beyond the natural precipitation, only irrigated during planting. The effect of water stress on the harvest is performed by comparative test observation, Brix°, and the measurement of the phytonutrient values already listed, which I concluded that industrial processing is required by the control group with the most advantageous properties, thus with group continuity and further experiments.

Fully ripe fruits with deep red color (Raw) were freshly harvested from at least 40 plants from the two locations in 4 replicates for each. Processing of tomato for juice extraction was carried out in the Department of Food Industry, Institute of Food Science and technology of the University of

Agricultural and Life Sciences (Budapest, Hungary). The processing included washing, shredding, cold-break extraction (CBE) hot-break extraction (HBE) and pasteurization. In the case of HBE, the shredded tomato batch was heated to 90 °C for 15 min, while CBE was performed at 60°C for 30 min using steam-heated, double jacket cookers. The pomace, which contains the peels and seeds from cold-break (CBP) and hot-break (HBP) extractions were separated by using rotatory roller sieves. The juices were packaged in plastic bottles and stored at -20°C when not immediately analyzed. In the case of CBE, the entire stock was heated to 60°C and stored as for the hot break-extracted samples. The fruits of the raw tomato materials (control) were homogenized in a warring blender and kept at -20°C until the analysis of carotenoids and tocopherols.

The extraction of carotenoids and tocopherols was carried out with a 1: 5 mixture of 60 ml of analytically pure methanol: 1,2-dichloroethane using a rotary distillation apparatus, while in the case of vitamin C, the extraction was performed with 3% metaphosphoric acid. A Hitachi Chromaster HPLC instrument consisting of a Type 5110 gradient pump, a Model 5430 diode sensor, and a Type 5440 fluorescence detector was used for the determination of different phytonutrients. Separation and data processing are performed by EZChrom Elite software. Simultaneous measurements of carotenoids and tocopherols were performed on a C-30 core 150 x 4.6 mm, 2.6µm column (Thermo Scientific, USA) with a gradient elution of (A) tert-butyl methyl ether (TBME) in 1% water in methanol (B).

Carotenoid compounds were detected between 195 and 700 nm. During the analysis of the control and the processed samples, I used a 35-min HPLC protocol, which separated the main carotenoids, geometric isomers or derivatives from the outside. Tocopherol compounds were detected between EX:295 and EM:325nm. I used a 35-min HPLC protocol and detection was performed with a FL (fluorescent) detector. Detection and identification have become even more accurate with the HPLC-MS / MS method we have developed (based on the total ion and its fragments m / z) (Daood et al. 2021).

Vitamin C (L-ascorbic acid) was separated on aqua C18, 3μ, 150 x 4.6 mm column (Nataulis from Machery Nagel, Düren, Germany), with a gradient elution of acetonitrile (A) in 0.01M KH_2PO_4(B). The separated compounds were detected by DAD between 190 and 400nm. Identification and quantification of L-ascorbic acid were based on calibration curve of standard material. Under the used conditions L-ascorbic acid had an absorption maximum at 262 nm, at which the area was integrated.

Results

Carotenoids

For carotenoids the chromatographic profile of tomatoes and manufactured products between five geometric isomers of lycopene, 2 β-carotene isomers and several mono- and di-epoxides of these carotenoids. In addition, γ-carotene, neurosporen, residual xanthine, or phytoene and phytofluene are also efficiently selected for the method used. The major derivatives of lycopene were the 9- and 13-cis isomers and epoxides, while the all-trans-β-carotene derivatives were dominated by epoxides. All-trans-lycopene accounted for 85–87% of the total carotenoid amount, a ratio that is consistent with literature data. It is of great importance that the HPLC-MS / MS method we developed was the first to detect dimethoxy lycopene and dihydroxy cyclo lycopene adduct in tomato products.

The results indicated that climate variables and other abiotic factors can affect the content and stability of phytonutrients of tomato products. The high temperature and low precipitation particularly at 3 weeks before harvest caused an increase in carotenoids content, of tomato with low stability during juice extraction. The inverse tendency was observed in tomato produced in area with lower temperature and higher precipitation during cultivation season. It seems that the interaction between some biochemical and physical factors could determine the tendency and magnitude of change of carotenoids during CBE and HBE. It is possible that the abiotic factors, particularly precipitation, may influence the availability of some microelements essential for the activity of enzymes involved in carotenoid biosynthesis and oxidation. The difference between the levels of nutrients in the soil required for the maximal activity of the enzymes responsible for the oxidative degradation of carotenoids may lead to higher concentrations of carotenoid derivatives in the cold-breaking juice. Because of the heat treatment at 90 ° C during hot-break (HBP) extractions technology is able to inactivate most enzyme systems, the increase in the amounts of cis isomers, epoxides, and hydroxylated derivatives is likely due to heat- and molecular oxygen-induced reactions.

Tocopherols

The HPLC provided a good separation of the main tocopherol analogs (α-β-ɣ-tocopherols), their esters and oxidized derivatives. The dominant compounds are α-tocopherol and its acylated ester and reduced α-tocopherol quinone (αTQH2), while ɣ-tocopherol is abundant in the pomace fraction as it distributes high proportion of seeds, the main source of ɣ-tocopherol.

Tocopherol content of raw tomato and juices in 2019 was higher than in 2018 irrespective of the cultivation location. As the number of days in excess at 30°C is substantially higher in 2019 than in 2018 in both locations it is evident that the concentration of tocopherol in tomato is temperature- and sunshine-dependent. In both cultivation seasons and location, homogenization and heating of tomato fruits resulted in a significant loss of both free and acylated α-tocopherol in juices as a great portion of the exocarp is removed with the pomace fraction. The difference in climate between the two locations manifested itself in changing the response of tocopherols to thermal processing. CBE of tomato cultivated at lower temperature and higher precipitation produced juice and pomace with higher tocopherol content as compared to that obtained with HBE ($p<0.01$) despite the effect of high heating temperature on release of the compounds from peel and seed membranes. The opposite trend was observed in juice from tomato cultivated with at temperature and low precipitation with which content of vitamin E components was higher in HBE. The response of tocopherols to thermal treatments was almost similar to that noticed for carotenoids.

The obtained results showed that the unesterified α-tocopherol content in the extracted tomato juice was lower than that of the acetylated esterified α-tocopherol. During the chemical volatilities that occur during the processing process, the acetylated derivative has greater chemical stability. In the case of pomace, the level of the oxidized form of α-tocopherol (α-tocopherol quinone) is inversely proportional to the α-tocopherol content, which confirms the latter's significant antioxidant activity.

Vitamin C

As being heat- and molecular oxygen- sensitive substantial loss was expected for vitamin C as a function of thermal processing. The vitamin C content of raw tomato was 1200-1300 μg/g dry weight. It is believed that differences in the sunshine period, precipitation, and temperature during plant growth and before fruit harvest might cause a great decrease to the ascorbic acid stabilizing factors. In addition to the thermal treatment affecting the stability of vitamin C and the drastic decrease in the water content of the products, abiotic factors with significant seasonal variations also contributed to the different reactions of the samples. All of these factors greatly influenced the stability of L-ascorbic acid. The number of days with a temperature above 32°C was less and the amount of precipitation higher in Szárítópuszta than Szarvas. Based on the factors affecting stability and content of vitamin C in tomato it can be said that one or more of these factors may stand beyond the dramatic decrease in thermally treated juices and pomaces in cultivation season of 2018. It is believed that differences in the sunshine period, precipitation, and temperature during plant growth and before fruit harvest might cause a great decrease to the ascorbic acid stabilizing factors.

Thesis and recommendations

1. I developed an HPLC-MS / MS method for the separation, identification and quantification of carotenoid dyes in tomato products, which was used to isolate and identify 33 carotenoid compounds including geometrical isomers and derivatives of the main carotenoids. method development assisted to detect and identify dihydroxy-cyclo-lycopene adduct and dimethoxy lycopene for the first time in fresh and processed tomato products, respectively.
2. With application of the developed HPLC-FL method, It was found that the main oxidation product of vitamin E (α-tocopherol) is α-tocopherol hydroquinone (α-TQH2) and not α-tocopherol quinone (α-TQ).
3. Based on the data measured in 2018 and 2019, it can be concluded that in the case of UG812 J hybrid industrial tomato, water stress influences the process of carotenoid synthesis during ripening, ultimately shifting the lycopene-β-carotene ratio, sometimes resulting in an increase in β-carotene content.
4. I found that the pre-harvest temperature and the amount of rain significantly affect the stability of phytonutrients during processing.
5. I have shown that in tomatoes grown with a high-water supply (even heavy rain) and low temperatures, the content of bioactive compounds, especially vitamin C, decreases, but their stability significantly decreases during processing.

Based on my results, it is recommended that the content values of industrial tomatoes and their products from different production areas be continuously monitored in order to select the most suitable method for processing in order to produce a product with a high nutrient content.

It would also be worth considering the more frequent use of the high tocopherol fraction of the pomace in processing foods. This increases the product range of foods with high antioxidant capacity.

In the future, it would be worthwhile to further investigate and analyze the two first-described carotenoid derivatives, the dihydroxy cyclo-lycopene adduct and dimethoxy lycopene, as these studies could yield a number of significant results. Measurements with α-tocopherol hydro-quinone, also described for the first time in tomato product samples, could provide countless new results in the future.

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