SZENT ISTVÁN UNIVERSITY

EVALUATION OF FRUIT FORMATION IN PROCESSING TOMATO, WITH SPECIAL REGARD TO THE NON-DESTRUCTIVE ANALYSIS (NIR) OF NUTRITIONAL CONTENT

DOCTORAL (PhD.) DISSERTATION THESES

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1. RESEARCH ANTECEDENTS AND THE DEFINED OBJECTIVES

1.1 The significance and antecedents of the research

Tomato is the most important vegetable species in the world, currently produced on 4,700 – 4.800 thousand hectares with a total yield of 180 million tonnes. About 25% of all tomato crops can be considered processing tomatoes; however, production has seen significant increases over the course of the past three decades. Tomato was grown on approximately 1,700 hectares in Hungary in 2018 with an approximate yield of 125 thousand tonnes. Its role in a healthy diet is extremely important; in addition to having a great taste, tomato also includes a number of antioxidant compounds with new positive health effects being discovered every day.

Of these, the role played by lycopene is worth emphasising, which is widely known, as publications have proven, to prevent cancer and have beneficial effects against cardiovascular diseases. In addition, an increasing number of studies are reporting about β-carotene, zeaxanthin, and phytoene, which are attributed especial importance as regards the healthy functioning of the eyes.

The processing industry is setting increasingly stringent requirements for producers, which are capable of greatly influencing purchase prices. These include the °Brix value, where producers might even be fined for failing to meet the minimum value of 5 °Brix.

From the aspect of economy, it is also very important to realise marketable yields, which value is currently around 65-70 tonnes/hectare, depending on production conditions; lower yields mean production generates losses.

The effects that the various tomato varieties, production technologies, and abiotic factors (e.g. precipitation, temperature, sunlight) can have on nutritional parameters and economy are also well known. It is a fact that an adequate water supply results in high degrees of yield increase, which is why an irrigation regimen planned with great precision has to play an important role in the technology. In addition to the above, the process of laboratory testing is also a decisive factor, as analytical results currently take days and they are very expensive. Near infrared reflectance spectroscopy is a possibility for conducting quick and non-destructive analyses; however, this requires correct calibration, the research for which is not yet fully fledged and extends to micron-sized components to a limited extent only.

The present doctoral dissertation seeks answers and solutions to the above topics, attempting to examine and analyse those in the broadest spectrum possible.

1.2 Objectives

I have set the following objectives:

- The use of various irrigation treatments to compare the yields, dry matter content, and carotenoid content and composition of two tomato hybrids (Uno Rosso F_1 and Strombolino F_1) that are significantly different from one another.
- An analysis of lycopene content and yield, with a special emphasis on the percentage and quantity of all-trans and cis-lycopene.
- The study of four, significantly different vintages, which include an exceptionally dry (2013) and an exceptionally precipitation-heavy (2014) year.
- The use of near infrared reflectance spectroscopy to examine the nutritional parameters of tomato fruit, especially regarding °Brix and carotenoid components.

2 MATERIALS AND METHODS

The experiments were conducted at the teaching facilities of the Saint Stephen University's Institute of Horticulture between 2012 and 2015. The four consecutive years were characterised by significantly different weather conditions. The study extended to two different varieties, Uno Rosso F_1, which has a traditional average fruit weight, and the cherry-type hybrid Strombolino. The individual treatments were applied under identical production technology parameters. Irrigation was started the second week after planting, and water was provided thrice weekly, depending on weather conditions. The following equation was used to calculate optimal water dosage: *daily irrigation water dose (mm) = daily median temperature ×0.2.*

After harvesting, the samples were grouped into marketable and non-marketable (cracked, damaged, sick) categories and then transferred to the Szent István University Regional Knowledge Centre for laboratory testing. Once in the laboratory, the °Brix values of the fresh samples were measured as soon as possible using a KRÜSS DR201-95 refractometer. A high-performance liquid chromatograph (Hitachi Chromaster) was then used to examine the carotenoid components, including total carotenoid content, lycopene, β-carotene, lycoxanthin, zeaxanthin, phytoene, and vitamin C.

Concurrently, a Perten DA7200 (Perten Instruments) near infrared spectrometer was used to examine the same samples to obtain a spectrum in the 950 to 1650 nm range for all samples. The instrument works by shining white light on a homogeneous sample, which leads to a part of the light being absorbed and the rest refracted. The refracted light enters a diffraction grating that separates the light by wavelengths. This breaks the white light into a rainbow of colours, which the detector is able to measure and turn into a spectrum. An Unscrambler 10.3 (Camo Software) statistical software was used to compare the resulting spectrum information with the analytical results to determine the wavelengths with the highest correlation. In the interest of effectiveness, the software was used to continue examining the data using spectrum transformation techniques, including first and second derivatives, multiplicative scatter correction (MSC), and standard normal variate (SNV). The correlations were determined with the partial least squares (PLS) regression method, which yielded the calibration and cross-validation results and errors.

3 RESULTS

3.1 Changes in marketable yield quantities in the four years (2012-2015)

The field tests involved the assessment of four significantly differing vintages, which included an extremely dry year as well as a year with significantly more precipitation than usual. Tomato producers realise financial profits if they are able to produce at least 65-70 tonnes/hectare of marketable yield; the goal is therefore to achieve higher yields. It can be established on the basis of my results that this target can only be met with irrigation; the average yield of the four years was only 25.89 tonnes/hectare in the control (unirrigated) Uno Rossi parcel and an even lower figure of 20.37 tonnes/hectare in the corresponding Strombolino parcel. However, the Uno Rosso F_1 stocks that received the optimal amount of irrigation water were able to meet these expectations in all years but one (2014), with an average yield of 26.41 t/ha; the average of the four years shows a yield of 78.43 tonnes/hectare. The corresponding figure for Strombolino F_1 is 45.08 tonnes/hectare (Table 1).

Table 1: Marketable fruit yields of Uno Rosso F_1 (UR) and Strombolino (STR) varieties by year and irrigation water quantities (2012-2015). K=control, with treatments involving 50% and 100% of required irrigation quantities (n=4)

	2012	2013	2014	2015
URK (t/ha)	47.02±5.59a	20.21±7.06a	17.60±4.80a	18.74±3.66a
UR50 (t/ha)	68.22±9.56b	56.63±6.60b	19.12±2.92a	60.20±1.82b
UR100 (t/ha)	118.43±4.59c	97.60±12.06c	26.41±5.50a	71.30±2.65c
STRK (t/ha)	25.42±8.45a	27.15±1.60a	15.10±6.80a	13.84±2.07a
STR100 (t/ha)	59.15±5.26b	50.35±5.87b	10.39±11.40a	60.45±2.92b

The values marked with different letters within the columns deviate significantly from each other at a probability level of $p \leq 0.05$, examined separately by variety.

3.2 °Brix and dry matter content

The °Brix value is one of the main pillars of processing industry requirements, which has to reach a minimum value of 5 °Brix, under which purchase prices may end up being reduced. The fact is that increased water quantities have a positive effect on yields but a negative effect on the °Brix factor. This exact result can be exactly quantified on the basis of the data of the examined four years: increases in irrigation water led to commensurate decreases in °Brix values. Variations are also quite significant between the vintages: there is a 39% difference between the highest (2012: 5.20 °Brix) and the lowest (2015: 3.73 °Brix) °Brix values in the optimally irrigated Uno Rosso F1 stock and a 43% difference in the Strombolino F1 variety (6.30 vs. 4.40 °Brix). The difference between the varieties was heavily in favour of the cherry-type Strombolino, which had 20% higher yields in all years except 2014. There was only one treatment where the yield achieved the economic threshold of 70 tonnes/hectare and the minimum value of 5 °Brix: in 2012, in the optimally irrigated Uno Rosso F_1 variety (Table 2).

Table 2: °Brix values by variety, vintage, and different irrigation quantities (n=4)

Treatments	2012	2013	2014	2015
URK	7.20±0.30a	6.20±0.59a	5.10±0.10a	8.03±0.26a
UR50	6.10±0.50b	5.00±0.40b	4.40±0.20b	5.02±0.44b
UR100	5.20±0.30c	4.80±0.50b	4.40±0.10b	3.73±0.15c
STRK	7.60±0.20a	6.60±0.88a	4.60±0.20a	7.35±0.10a
STR100	6.30±0.30b	6.00±0.61a	4.40±0.00a	4.53±0.33b

The values marked with different letters within the columns deviate significantly from each other at a probability level of $p \leq 0.05$, examined separately by variety.

The Brix yield value is representative of the quantity of dry matter harvested from one hectare and can be calculated on the basis of the marketable yield and the °Brix value.

The data in Table 3 show a similar tendency in the vintages than experienced in the °Brix value; in this case, the difference between the highest and the lowest values obtained by the Uno Rosso F_1 variety (5.10 t/ha vs. 2.10 t/ha) is more than double and is even more, more than threefold in the case of Strombolino F_1 (4.97 t/ha vs. 1.30 t/ha). When examining the dry matter content harvested from one hectare, Strombolino F_1 shows no significant deviations compared to Uno Rosso F_1, based on which it can be determined that the higher °Brix value is able to compensate the lower yield quantity.

Table 3: Brix yield (t/ha) by vintage and different irrigation quantities (n=4)

Treatments	2012	2013	2014	2015
URK	2.12±0.25a	1.60±0.51a	1.50±0.20a	1.17±0.19a
UR50	3.45±0.44b	2.61±0.51b	1.60±0.20a	2.83±0.22b
UR100	5.10±0.49c	4.35±0.65c	2.10±0.10b	2.55±0.23b
STRK	2.94±0.36a	1.70±0.22a	1.20±0.40a	1.07±0.10a
STR100	4.97±0.43b	3.20±0.60b	1.30±0.30a	2.93±0.33b

The values marked with different letters within the columns deviate significantly from each other at a probability level of p≤0.05, examined separately by variety.

3.3 Total carotenoid content

Of phytonutrients, the value of total carotenoid content determines the total quantity of the more than 60 types of carotenoid compounds found in tomato. No clear correlation between the various vintages and treatments can be determined on the basis of the data; irrigation sometimes had a positive and sometimes a negative effect on its quantity.

Table 4: Total carotenoid content in the years examined (2012-2015) (n=4)

Treatment/Year	2012	%	2013	%	2014	%	2015	%
URK (µg/g)	116.98±2.46a	100	80.32±3.59a	100	40.89±1.63a	100	137.37±6.32a	100
UR50 (µg/g)	128.80±3.25b	110	98.24±2.22b	122	41.19±1.22a	101	138.10±5.63a	101
UR100 (µg/g)	116.92±5.21a	100	90.44±2.36c	113	31.85±1.45b	78	94.27±4.85b	68
STRK (µg/g)	168.67±1.69a	100	121.16±1.22a	100	64.14±3.15a	100	n.a.	n.a.
STR100 (µg/g)	159.94±2.47b	95	126.56±2.49b	104	56.05±4.56b	87	n.a.	n.a.

The values marked with different letters within the columns deviate significantly from each other at a probability level of p≤0.05, examined separately by variety.

However, the difference between the varieties showed a significant deviation. Depending on vintage, in the case of the optimally irrigated treatments, Strombolino F_1 contained 36-75% more carotenoids than Uno Rosso F_1, which tendency is also similar to the control stock (Table 4).

3.4 Lycopene content and yield

Lycopene makes up the majority of total carotenoid content. In the case of Uno Rosso F_1, the lycopene content of the fruit varied between 27.03 and 114.90 µg/g, which is almost a 4.5-fold difference. As regards the results, an important factor is the percentage of all-trans and cis-lycopene, as the cis isomer can be much better utilised by the human body. The all-trans form is transformed into the cis-lycopene form as a result of heat, which is why its percentage in processed industrial products (ketchup, tomato juice) is significantly higher. The quantity and percentage of tomato meant for fresh consumption is changeable and varies between varieties and treatments, in addition to which it is also greatly affected by weather conditions. In the case of Uno Rosso F_1, the all-trans to cis ratio was 2% in 2012 but was 19% in 2015 in the optimally irrigated treatment (Table 5). This difference is attributable to the pre-harvest condition, as in 2015 a strong warm front arrived in the last phase of ripening (which is the most crucial from the aspect of lycopene production).

Table 5: Lycopene content by year and irrigation quantities (Type: Uno Rosso F_1) (n=4)

Year	Treatments	All-trans lycopene (µg/g)	Cis-lycopene (µg/g)	Σ Lycopene (µg/g)
2012	**Control**	103.62±1.14a	2.09±0.47a	105.71±.0.86a
	100	104.81±8.46a	2.32±0.34a	107.13±4.65a
2013	**Control**	68.96±16.82c	0.78±0.53b	69.74±8.62c
	100	76.02±3.06c	1.91±1.07ab	77.93±2.06c
2014	**Control**	33.90±1.97d	1.93±0.26a	35.83±1.12e
	100	25.18±6.09d	1.85±0.33ab	27.03±3.21f
2015	**Control**	100.62±1.97a	14.28±0.64c	114.90±1.31g
	100	66.10±7.24c	12.68±1.87c	78.78±4.55c

The values marked with different letters within the columns deviate significantly from each other at a probability level of $p \leq 0.05$.

A similar tendency can be seen in the case of Strombolino F_1, where the lycopene content varied between 46.01 and 150.20 µg/g depending on vintage and irrigation. However, the all-trans to cis ratio is more beneficial than in the case of Uno Rosso F_1; for example, in 2013 its value was 7.2% in the control stocks (Uno Rosso F_1: 1.1%) and 8.3% in the irrigated stocks (Uno Rosso F_1: 2.5%) (Table 6).

Table 6: Lycopene content by year and irrigation quantities (Type: Strombolino F_1) (n=4)

Year	Treatments	All-trans lycopene (µg/g)	Cis-lycopene (µg/g)	Σ Lycopene (µg/g)
2012	**Control**	142.18±3.95a	8.02±1.89a	150.20±2.92a
	100	133.25±2.75b	7.45±0.65a	140.70±1.70b
2013	**Control**	94.47±6.99c	7.35±3.40a	101.82±5.19c
	100	100.34±3.13c	9.05±4.71a	109.39±3.92c
2014	**Control**	51.48±8.14d	2.03±0.68b	53.51±4.46d
	100	42.91±1.81d	3.10±0.36c	46.01±1.08e
2015	**Control**	n.a.	n.a.	n.a.
	100	n.a.	n.a.	n.a.

The values marked with different letters within the columns deviate significantly from each other at a probability level of $p \leq 0.05$. (n.a. = no data available)

The difference between the varieties is thus apparent in the all-trans to cis ratio as well as in the total lycopene quantity, of which Strombolino F_1 has significantly more than Uno Rosso F_1, which statement is true for all years and treatments.

As regards lycopene yields (Table 7), both varieties experienced the best results in 2012; in the case of irrigated stocks, this value was 9.73 kg/ha for Uno Rosso F_1. The various vintages were quite different from each other: lycopene quantity was only 0.65 kg/ha in 2014, with neither treatment reaching the value of 7 kg/ha in either of the remaining years. Despite the higher lycopene values among varieties, Strombolino F_1 was still unable to provide the same level as its more traditionally fruited friend, which yielded significantly higher yields in the case of the optimally irrigated treatments, with an average of 29% in the three years examined.

Table 7: Lycopene yields (kg/ha)
(n=4)

Treatments	2012	2013	2014	2015
URK	3.05±0.12a	1.33±0.06a	0.51±0.09a	1.67±0.13a
UR50	6.61±0.34b	4.67±0.30b	0.60±0.06a	6.60±0.22b
UR100	9.73±0.26c	6.70±0.33c	0.65±0.14a	5.33±0.19c
STRK	2.37±0.09a	2.70±0.11a	0.72±0.12a	n.a.
STR100	8.44±0.25b	5.32±0.22b	0.44±0.06b	n.a.

The values marked with different letters within the columns deviate significantly from each other at a probability level of $p \leq 0.05$, examined separately by variety. (n.a. = no data available).

3.5 The evaluation of near infrared reflectance spectroscopy results

The near infrared reflectance spectroscopy involved 64 samples in 2012 and 120 samples in 2013. The parameters examined included °Brix, acid and sugar content, and the various carotenoids. Not many articles have been written concerning the NIR-based analysis of carotenoids; those that exist used only a small sample size (30-50) or examined only one or two components. Contrarily, the present research involved a greater number of samples and compounds, including all-trans and cis lycopene, β-carotene, lycoxanthin, and zeaxanthin. The strongest correlation was found between β-carotene (R^2_{cv} = 0.89, RMSECV = 0.17) and lycoxanthin (R^2_{cv} = 0.84, RMSECV = 0.28) in the case of cross-validation; however, the results of the other components can also said to be suitable (Table 8). Based on the results obtained, it can be determined that near infrared reflectance spectroscopy is suitable for examining carotenoid-type compounds and °Brix values in tomato with a low error rate.

Table 8: The results of the calibration set based on 120 samples

	°Brix	All-trans lycopene	Cis-lycopene	Lycoxanthin	Zeaxanthin	β-carotene
Spectrum transformation	1st derivative + MSC	1st derivative	1st derivative + MSC	1st derivative + MSC	1st derivative + MSC	2nd derivative
Wavelength	950-1552	950-1650	950-1650	950-1650	950-1650	950-1300
R^2_C	0.84	0.78	0.83	0.88	0.83	0.90
RMSE (μg/g)	0.47	6.68	0.23	0.25	0.09	0.17
R^2_{CV}	0.77	0.75	0.76	0.84	0.75	0.89
RMSECV (μg/g)	0.55	6.88	0.27	0.28	0.10	0.17
PC	6	2	8	7	7	4

R^2_C: R^2 calibration; R^2_{CV}: R^2 cross-validation; RMSE: root-mean-square error; RMSECV: root-mean-square error of cross-validation, PC: main component number

3.6 New scientific results

1. Based on the 4 vintages, the numerical value of the extent to which changes in environmental factors (water supply, temperature) had a significant effect on the quantitative and qualitative parameters of the yield was calculated.
2. It was determined on the basis of the results of the examined two hybrids and four years that of the twenty irrigated treatments, the °Brix value met the canning industry requirement of exceeding 5 °Brix in thirteen cases.
3. It was determined that the lycopene composition, in light of the cis-lycopene concentration, is significantly higher if maximum temperatures exceed 30°C in the week prior to harvesting. This finding is important from the aspect of nutrition and physiology.
4. It was determined that the 950-1650 nm range of near infrared reflectance (NIR) spectroscopy, with the help of the appropriate spectrum transformation techniques, is suitable for determining the °Brix (R^2_{cv}=0.81) and lycopene (R^2_{cv} =0.84) values that are so important to the processing industry.

4 CONCLUSIONS AND RECOMMENDATIONS

The experiments involved studying various parameters of processing tomato under significantly variably environmental conditions over a period of four years, which included drought and a year with significantly higher than average precipitation. In connection with climate change, climatologists mention the frequency of extremes, meaning average years may be increasingly rare in the future. In Hungary, chances will increase that very hot and very cold years will both occur. This is a significant natural risk for processing tomato production.

The first part of the present research was aimed at studying yield quantities, in connection with which it was determined that there was a significant deviation both as regards the examined varieties and the irrigation treatments. The effects of the individual years were quite apparent: more than four times the yield was collected in 2012 than in 2014 from the irrigated plots. The control plots did not even approximate the threshold values that can be said to be economical. Uno Rosso F_1, which has a traditional average fruit weight, realised significantly higher marketable yields in all four yields than the cherry-type Strombolino F_1.

As regards the °Brix value, it can be determined that there is a substantial difference between the individual years both in the irrigated and the control stocks; the difference between the highest and lowest values was 1.5 °Brix in the case of Uno Rosso F_1 and close to 2 °Brix in the case of Strombolino F_1. Of the two varieties, the cherry-type hybrid provided significantly higher °Brix values than its traditionally weighted counterpart, amounting to 17% over the average of the 4 years. As the cherry-type has higher °Brix values, there were no significant deviations in the varieties' Brix yields.

The examination of total carotenoid content did not show a clear correlation between the various irrigated treatments; however, it did identify a correlation between the varieties: Strombolino F_1 contained 35-40% more.

Based on the obtained results, it can be determined that lycopene is responsible for a significant portion of the total carotenoid content; accordingly, the results were similar in their tendencies. The effects of the different years was apparent, with a four-fold difference measured between the weakest year of 2014 and the best year of 2012 in the case of the optimally irrigated treatments. Among the varieties, Strombolino F_1 provided 30-70% better results in the years examined. However, Uno Rosso F_1 showed significantly higher lycopene yield results in the case of all treatments.

Findings similar to lycopene were made regarding the other studied carotenoids as well, i.e. Strombolino F_1 contained significantly higher amounts than Uno Rosso F_1, and there were substantial differences between the various years, with 2014 again providing exceptionally low figures.

When examining vitamin C, it can be determined that the different years had a strong effect on quantities, with a more than two-fold difference between 2012 and 2015. It can also be established that there was a significant difference between the two examined hybrids: in 2013, the difference in vitamin C content was more than two-fold in the case of the optimally irrigated treatments.

The results of near infrared reflectance (NIR) spectroscopy show that the 950-1650 nm wavelength range is suitable for measuring °Brix (R^2_{cv}=0.81), acid (R^2_{cv}=0.63), sugar (R^2_{cv}=0.61) , and carotenoid-type compound (lycopene: R^2_{cv} =0.84) content with a low error rate. The various spectrum transformation techniques can be used to further confirm the correlations.

5 PUBLICATIONS RELATED TO THE DISSERTATION TOPIC

Articles in IF and SCI periodicals

Deák, K., Szigedi, T., Pék, Z., Piotr, B., Helyes, L. (2015): Carotenoid determination in tomato juice using near infrared spectroscopy. **International Agrophysics** (IF:1,14) 29: 275-282.

Györé Kis., Gy. Deák, K. Lugasi, A. Csúr Varga, A. & Helyes, L. (2012): Comparison of conventional and organic tomato yield from a three year-term experiment. **Acta Alimentaria**, (IF: 0,39) 41 (4): 486-493.

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Deák, K., Varga, A., Lugasi A., Helyes, L. (2012): Az ökológiai és a konvencionális termesztésű paradicsom egyes beltartalmi összetevőinek összehasonlító vizsgálata. **Kertgazdaság**, 44 (2): 3-8.

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Deák, K., Szigedi, T., Pék, Z. (2015): Az ipari paradicsom minőségének meghatározása közeli infravörös spektroszkópiával. **Kertgazdaság,** 47 (1): 3-7.

Deák, K., Égei, M. (2020): Vízellátás hatása ipari cseresznye paradicsom termésképzésére és a fontosabb fitonutrienseire. **Kertgazdaság**, 52 (3): 17-26.

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Deák, K. J., Daood, H., Neményi, A. (2013): Effect of elevated potassium supplement on yield formation in case of processing tomatoes. 12th Alps-Adria Scientific Workshop, **Növénytermelés/Crop production** 62: 63-66.

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Deák, K. J., Varga A., Lugasi A., Helyes L. (2012): Az ökológiai és konvencionális termesztésű paradicsom egyes beltartalmi összetevőinek összehasonlító vizsgálata [In: Takács, M. (szerk.)]. **II. SzaKKKör Konferencia – Szakkollégiumok Konferenciája a Környezet- és Természetvédelemért**. 29.

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