SZENT ISTVÁN UNIVERSITY

FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, GÖDÖLLŐ

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Quality components of chili pepper determined by different coloured shading nets, genotypes and ripening stages

Ph.D. thesis

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1. Introduction, objectives

Chili peppers (*Capsicum spp*.) belong to *C. annuum*, *C. baccatum*, *C. chinensis*, *C. pubescens* and *C. chacoense* species, having highly pungent pods, but differ from Hungarian paprika or pungent bell peppers (Hayano-Kanashiro et al. 2016). Chili peppers are horticultural commodities of special interest, which can be consumed fresh or processed. Peppers are good sources of nutritionally important phytonutrients such as vitamin C, vitamin E, vital carotenoids, polyphenols and, moreover, the sole source of capsaicinoids (Arimboor et al. 2014).

The level of various secondary metabolites, such as capsaicinoids and ascorbic acid, in peppers is affected to a high extent, by the UV light reaching the plants (Wink, 1988; Namdeo, 2007), high exposition to sunlight (Rao és Ravishankar 2002), water avabiality (Valiente-Banuet és Gutiérrez-Ochoa, 2016), harvest times (Kim et al. 2008), post harvest processes (Lee és Kader 2010) and net shading (Selahle et al. 2014).

Application of net shading is commonly used in bell pepper production in areas suffering from high exposition of sunlight. For instance, in the mediterranean region of Europe (Legarrea et al. 2010), in Serbia (Ilić és Fallik 2017), in Izrael (Shahak 2008), in Brasil (Ferreira et al. 2015), in the south part of the US (Masabni et al. 2016), in India (Nangare et al. 2015) and in Hungary as well (Ombódi et al. 2016). Net shading is used to modify the energy of light, thus affecting the plant's light-regulated processes. Unfortunatelly it is not clearly understood how certain agricultural practices, in particular net shading, influence content and composition of quality components and nutrients such as capsaicinoids, ascorbic acid, carotenoids and others.

Another important question in pepper production and processing is how the phytonutrients change with ripening. The dynamics of ripening is well investigated for a long time, but the results are still not in agreemant with each other, most likely due to variation between genotypes and uncertanity of ripening stages.

Objectives of the study:

- to study the phytonutrients and quality determining compounds of chili pepper with modern liquid chromatographic methods;
- to compare the different genotypes of chili (*Capsicum frutescens* and *Capsicum annuum*) with special regard to ripening dynamics of capsaicinoid, polyphenol and Vitamin C;
- to investigate the simultaneous effect of different coloured shading nets and harvest times on the capsaicinoid and Vitamin C content of 'Fire Flame', 'Star Flame' and 'Jalapeno' (*C. annuum*) and on the carotenoid composition of 'Fire Flame' as well;
- to highlight on the effect of different coloured shadings nets on marketable yield and photosynthetic activity of 'Fire Flame', 'Star Flame' and 'Jalapeno' (*C. annuum*) genotypes of chilli crop.

2. Material and Methods

Four separate experiments were conducted in the research field of Szent István Univeritsy during 2013, 2014 and 2015 cultivation seasons.

- In 2013 four genotypes of chili ('Bandai', 'Beibeihong 695', 'Lolo 736' és 'Chili 3735') were transplanted to the open field and harvested on September13th.
- In 2014 the indoor cultivaton was made in „Soroksár 70" type plastic house. Transplantation was made on April 29th in a plant density of 4.16 plant/m^2. Harvest times schedulded on July28th, September 16th and October26th.
- In 2014, in outdoor cultivation, the peppers were transplanted on May 19th with a plant density of 4.16 plant/m^2. Harvest times were schedulded to be on August 24th and September 28th.
- In 2015 only indoor experiment was conducted in a „Rischel 8" type double covered plastic house. Harvest times were schedulded to be on July 27th and September 28th.

The plants were shaded in 2014 and 2015 with green, white (Első Magyar Kenderfonó, Szeged) and red nets (Ginegar, Izrael) wit unshaded plants as control. All shading nets were placed perpendicular to the twin rows over a space 8 m in width, thus creating a block under which four biological replicates were randomly appointed. Ten plants were selected from each replicate and subjected to different measurements such as chlorophyll fluorescence and market-quality pepper yield, and determination of content of phytochemical during the growing season. The shading experiment were only conducted on 'Star Flame' and Fire 'Flame' in the first two seasons and in 2015 'Jalapeno' genotype was included. Two *Capsicum annum* L. hybrids, each with a distinct morphology, were investigated in this study: "Star Flame", which produces fruit approximately 12 cm in length that develop vivid yellow coloring upon maturation, and "Fire Flame", which is characterized by fruits having 14–15 cm length and possess a deep red color. Seeds for these two hybrids were purchased from Seminis® (Kecskemét, Hungary).

Determination of phytonutrients were carried out by HPLC (Hitachi, Chromaster) located in the Regional Knowledge Centre, Gödöllő). The compounds were separated on analytical columns containing C18 endcapping. The capsaicinoids were detected by fluorescence detection (extintion: 280 and emission: 320 nm), the other phytonutrients were detected by a diode array detection system between 200 to 500 nm. The qualification of the various phytonutrients were done by comparing their spectral charcteristic and retention times with available standard materials and literature data. The different compounds were quantified by injection of standard material with known concentrations .

Functional group analysis was used to organize the huge number of compounds. Total capsaicinoid (TC) was calculated as the sum of the individual compounds that appeared on the chromatogram, as follows: nordihydrocapsaicin (NDC), capsaicin (CAP), dihydrocapsaicin (DC), and other minor components (homocapsaicin derivatives and homodihydrocapsaicin derivatives).

Bpth yellow and red Carotenoids were arranged according to their esterification levels as follows: free pigments, mono-esteres (ME), β-carotene and diestres (DE).

Statistical analyses were performed using IBM SPSS 22 software (IBM, USA). The effect of net shading on market-quality pepper yield was analysed using a linear model, in which pepper yield and shading were considered as the dependent variable and the explanatory variable, respectively. The effect of shading on chlorophyll fluorescence (Fv/Fm) and marketable yield was calculated by one-way linear model. Pairwise comparisons were made via Tukey HSD post-hoc test. The nutritional contents of hybrids were examined separately because such properties are considerably different in red and yellow pepper cultivars as demonstrated by previous studies (Ghasemnezhad et al. 2011). Throughout the study, α was set to 0.05.

3. Obtaied Results

Altogether 6-7 capsaicinoids, cc. 60 carotenoids (arranged in functional groups based on esterification and colour), 9 polyphenols were detected in 7 different genotype. The most pungent genotype was 'Bandai' in green ripening (1176.1±112.1 μg/g), and the one with the highest Vitamin C content was 'Fire Flame' (3689.4±160.61 μg/g). The study on the ripening process shows that capsaicinoid content is decreasing (expect in 'Beibeihong') and Vitamin C is increasing with ripening. In 'Fire Flame hybrid, the highest Vitamin C was measured at colour break stage (2691.2±40.95 μg/g).This tendency has been observed in Hungarian spice paprika as well. Most of the major polyphenols increased during ripening except for vanillic acid derivative, which tended to decrease after green stage. The dominant polyphenol compound was naringenin-diglucoside, maximizing at red ripening stage in 'Bandai' (368,8±30,77 μg/g).

1. New scientific result (thesis)

By using recent liquid chromtographic methods that in the investigated I proved that in 7 genotypes of chilli, the capsaicinoid content is maximized in the biologically mature green pods expect in 'Beibeihong', therefore, so to achieve the highest yield of capsaicinoids the pepper should be harvested at green stage of ripening.

In the outdoor cultivation (in 2014) net shading significantly affected ($p<0.001$) the Vitamin C content of 'Star Flame'; the unshaded control yielded fruits with higher vitamin C content than that found in fruits cultivated under red and white shadings in both harvests. The effect of harvest time was also significant ($p<0.001$), the maximum value was found at the second harvest under the unshaded control conditions (3220±185 μg/g). In case of 'Fire Flame'the interaction of the two factors showed significant influence on vitamin C ($p=0.046$), the higest average value was in the same combination of second harvest and non-shading as in 'Star Flame'. It has been proved that in sweet bell pepper the unshaded comparing to shaded peppers gives higher Vitamin C content (Frezza et al. 2016). Based on our measurements the cummulated average temeperature (18°C) during the intense fruit ripening period (14 days) before the August harvest and the maximal daily degrees (26-29°C) proved to be too high and thus inhibited Vitamin C synthesis, so the autumn harvest resulted in a higher vitamin V levels.

2. New scientific result (thesis)

I stated that the use of coloured shading nets in outdoor cultivation decreased vitamin C concentration in both 'Star Flame' and 'Fire Flame', and moreover, vitamin C content of 'Fire Flame' is prominent (3748±121 µg/g) without shading.

In 2014, indoor experiment (Soroksári 70 type plastic house) the Vitamin C content of 'Star Flame' was significantly influenced by the interaction of shading and harvest time ($p=0,004$). In case of 'Fire Flame" the harvest time had significant influence on vitamin C content ($p<0,001$). In 2015 (Rischel type plastic house) in case of 'Star Flame' the shading ($p=0,001$) and harvest time ($p=0,002$) had significant effect on vitamin C level. In case of 'Fire Flame' there was significant influence of shading ($p=0,006$) and harvest time ($p<0.001$). In summary, the results of both plastic house experiments, the white shading is the most sufficent shading to improve Vitamin C content. Mashabela et al. (2015) stated that in plastic house the pearl net induced the highest Vitamin C value due to high transmitted radiation in the red and infrared wavelenghts in bell pepper. The white shading net allowed a higher transmittance in the UV region than red or green shading net and that induced the synthesis of phytonutrient in general (Wink, 1988; Namdeo, 2007).

3. New scientific result (thesis)

I stated that white net shading is the most effective to incerase Vitamin C value in plastic house cultivation.

In the outdoor experiment (2014) total capsaicinoid (TC) of 'Star Flame' was significantly influenced by harvest time ($F_{1,24}=56.107$, $p<0.001$). For 'Fire Flame' the interaction of harvest time and net shading had the highest significant effect ($F_{3,24}=8.155$, $p<0.001$).
Between the harvest time there was a 1.5 times difference in TC value of 'Fire Flame' which means it was the main factor. In both genotypes the harvest on 28th September resulted in a higher TC, so 14°C as cummulated average temperature and lower radiation promoted the TC synthesis. It was found that the average of minimum temperatures in the last 2 week before harvest was 6°C including days with 2-3°C as minimum scores. For pepper, which requires 25°C for optimal growth, this low temeprature was a serious stressor and the plant responded wih elevated TC. Gurung et al. (2011) found lower temparature and radiation induces pungency. The minimum temeparture before the September harvest went below 5°C, according to Otha (1960) lower night temparatures indiced higher pungency.

4. New scientific result (thesis)

I stated, that the cold period (2-3 °C daily minimum) before the September harvest stimulated capsaicinoid synthesis in both ’Star Flame’ and ’Fire Flame’.

The Fv/Fm value is an indicator of plant stress, a plant cultivated in optimal environment has its Fv/Fm value in a narrow range (Bolhar-Nordenkampf et al. 1989). In the openfield experiment there was a significant and negativ correlation beetwen TC and Fv/Fm in both ’Star Flame’ (Pearson=-0.66, $p<0.01$, N=32) and ’Fire Flame’ (Pearson=-0.74, $p<0.01$, N=32). In the plastic house experiment (2014) in ‘Star Flame’ (Pearson= -0.45, N=48, $p<0.001$) and in ‘Fire Flame’ (Pearson= -0.45, N=48, $p<0.001$) this relation was weaker.

5. New scientific result (thesis)

I stated, that there is a negativ and significant correlation beetwen TC and Fv/Fm in both ’Star Flame’ and ’Fire Flame’ and this relation is weaker in the plastic house than in the openfield.

4. Discussion and conclusion

The first objective of my work was to investigate capsaicinoid, polyphenol and vitamin C content in seven hybrids of chili and characterisation of ripening stages was also aimed. We concluded that pungency decreases with ripening and Vitamin C and carotenoid+chlorophyll content increases. The latter value is very low in the green ripening stage and than a great increase was observed in the colour-breaking stage and then a gradual increase was measured. In ’Fire Flame’ and ‘Jalapeno’ the highest Vitamin C was measured int the colour breaking stage. The most polyphenol compounds also increased, but vanillic acid derivative decreased after green stage. We suggest that the utilisation should determine the harvesting stage, eg. if maximal capsaicinoid content is aimed the peppers should be harvested at green stage, if the maximal vitamin C, carotenoid or polyphenol content is the main target, the pods should be harvested at red ripening stage.

The second objective of the study was to investigate the simultaneous effect of harvest time and net shading on Vitamin C content. In both plastic house experiments we stated that the white net shading stimulates it most effectively, because it doesn’t selects in the quality of the incident light (in wavelenghts) and doesen’t decreases the quantity of the incident light comparing to red or green net shading. If the high Vitamin C value is the aim of cultivation, the white net shading is recommended in plastic house cultivation.

Another objective was to investigate the simultaneous effect of harvest time and net shading on total capsaicinoid (TC). We observed that TC synthesis is most inhibited by the red coloured net comparing to unshaded control, the white net’s effect is similar to the unshaded control. The effect of green shading is most variable, because in some cases, it significantly increased (2014, indoor cultivation ‘Star Flame’ September end harvest and in 2015 indoor cultivation ‘Jalapeno’ September end harvest), but in other cases it did not alter as compared to the unshaded control.

According to the openfield experiment the harvest time has a great influence on TC. We found that the average of minimum temperatures in the last 2 weeks before harvest was 6°C that included days with low (2-3°C) minimum temperature. For pepper (which requires 25°C for optimal growth) this low temepratute was a serious stressor, and the plant responded wih elevated TC. We concluded, that more pungent peppers could be harvested in Autumn after a relatively cold period.

In the plastic house ('Rischel type', 2015) the effect of harvest time was more prononced, but it was caused by the 50% decreased amount of irrigation from the begining of September. This stressor stimulated the TC values and the 'Fire Flame' become almost as pungent as 'Star Flame'. The effect of cummulated radiation before September harvest on TC of 'Fire Flame' was significant ($r^2=0.501$, $p=0.002$, $y=0.70\times-155.72$).
To have a more accurate insight how different coloured shading nets influence pungency, we would need to perform an experiment in phytotrons, where all environmental factors are regulable (temperature, the amount of radiation) and thus only the corresponding wavelength of the exact colour would be set as variable.

Another objective was to investigate the simultaneous effect of harvest time and net shading on carotenoid groups of 'Fire Flame'. In both openfield and platic house it was observed that the harvest on end of September resulted in the highest carotenoid values, because the daily maximal temperatures measured before the August and July harvest (26-29°C) inhibited carotenoid synthesis. Mostly the coloured net shading decreased carotenoid values.
The highest levels of Zeaxanthin and β-carotene, the biologically most active phytonutrient among the investigated carotenoids were achieved at the last harvest (September or October) without shading. From the point of view of processing red ME and red DE values are the most important. In the plastic house experiments such comonents were stimulated in the August harvest. The highest average of red and yellow rate was observed under the green shading in all three experiments that indicates most colour stability.

The effect of net shading on marekatble yield was the same among genotypes, but different between years. In 2015, the red net decreased the yield compared to unshaded control and in 2014 the white net had favourable effect on yield compared to unshaded control. The decreasing effect of red net was due to the combined effect of the double covered 'Rischel type' plastic house and red net. In Hungary the green net shading is the most commonly used, but it did not cause additional marketble yield in both experiments.
According to the experimental data of 2014, the Fv/Fm value was suboptimal under the unshaded control, because in the lack of protection the plants evaporate more and this leads to a water stress that manifested itself in decreased Fv/Fm values. Also in 2014 significantly

negativ correlation was observed beetwen TC and Fv/Fm. We concluded that lower photosyntetic activity corresponds to higher pungency, and this relation is more pronounced in openfield than plastic house cultivation.

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