ANTIOXIDANT CAPACITY OF *CAPSICUM* GENOTYPES EXHIBITING DIFFERENTIAL LILAC COLOURATION DURING MATURITY

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Abstract

Total phenolic content (TPC) and antioxidant activity of 7 coloured *Capsicum* genotypes in four different maturity stages were investigated. The total phenolic content of the genotypes determined by the Folin-Ciocalteau method was in average higher in the early phenophases than in the fully ripe phase. Among the genotypes the extreme lilac exhibited the highest amount of TPC as a result of the putative additive effect of the high levels of anthocyanins. On dry weight basis there was a 4.5-fold difference at the ripe stage in between the extreme lilac and the white berry genotypes. Antioxidant capacity of the samples during ripening followed a similar pattern than the TPC and the values showed positive correlation from green stage 2 onwards. The study indicates that anthocyanin pigmentation could add up to the functional properties of the peppers.

Introduction

Pepper (*Capsicum annuum* L.) is one of the most commonly used spice and vegetable crops worldwide. Besides its shape and pungency, colour is a commercially important attribute as well [1, 2]. Fruit colour is of upmost importance since the pigments affecting the colouration further contribute to nutrition and flavour. The main determinants of pepper fruit colour are the chlorophyll, carotenoid and anthocyanin pigments [3]. Anthocyanins comprise a major branch of the phenylpropanoid pathway and were proved to possess - beside numerous other pharmaceutical applications to human health - antioxidant qualities. From nutraceutical aspects, various pepper fruits' carotenoid accumulation is among the highest in plants [4]. A teaspoon of its powder provides the Recommended Dietary Allowance for vitamin A of an adult person after the conversion of the precursor carotenoids to vitamin A [5]. In addition, they also act as antioxidants and free-radical scavengers, thus reducing the risk of several diseases such as cardiovascular or neurological disorders [6]. Besides phenolic compounds and various carotenoid pigments, peppers are also an excellent source of ascorbic acid.

Colour of the immature pepper fruits varies from ivory to green, with different shades of violet to nearly black [7]. Whereas the colouration of mature fruits of cultivated peppers ranges from white through yellow, orange and red to even brownish hue [8]. In most cases the presence of both chloroplhylls and anthocyanins in the berry is transient as they are gradually degraded during ripening as the level of carotenoids rises [9]. Just like the extent of pigmentation, the level of antioxidants can vary with plant parts, phenophase and with post-harvest handling [10].

Since these compounds exhibit antioxidant activities it is supposed that different genotypes at different phases of maturation have distinct antioxidant capacity. Here we describe the total phenolic content and the antioxidant activity of 7 genotypes displaying differing degrees of anthocyanin pigmentation.

Material and methods

Plant material

Seven mutant genotypes of *Capsicum* species were obtained from PepGen Ltd. All plants received similar water and fertilizer dosages and were kept under greenhouse conditions. Samples were collected at four different stages of ripening, 2 unripe stages, turning phase and at biological ripe phase. Samples were stored at -70 ^{0}C until analysed (Tab. 1, Fig. 1).

Tab. 1 Genotype colour changes during maturity

Genotype	Unripe colour	Ripe colour
Sample 1	Purple	Purple
Sample 2	Lilac	Red
Sample 3	Lilac	Yellow
Sample 4	Striped lilac	Red
Sample 5	White	Red
Sample 6	White	Red
Sample 7	White	Red

Fig 1 Examples of the genotypes used, upper left: sample 4, upper right: genotype 7, lower left: genotype 3, lower right: genotype 2

Antioxidant activity

The plants antioxidant capacity was measured by the FRAP (Ferric Reducing Ability of Plasma) assay according to Benzie and Strain (1996) at λ=593 nm. Peppers were crushed and homogenized in liquid nitrogen, the extraction was carried out with MEOH:water:HCOOH (60:39:1 v/v %). Samples were centrifuged at 4 ^{0}C for 20 minutes resulting the supernatant that was used for the analyses. The results are expressed as mmol ascorbic acid equivalent per 1 g dry weight.

Total phenol content (TPC)

Total soluble phenols were measured with Folin-Ciocalteau reagent according to Singleton and Rossi (1965) at λ=760 nm. Sample preparation was the same as above. The results are expressed as mmol gallic acid equivalent per 1 g dry weight. For both assays laboratory duplicates from each sample were used.

Results and Discussion

The nutraceutical properties make phenolics an indispensable element of any healthy diet. There is an increasing trend towards the breeding of genotypes with elevated nutrient content, therefore the quantification of phenolics in different genotypes in different phenophases could aid the breeding works of functional foods. Peppers are an optimal source of nutraceutical compounds which are mainly localized in their peels thus through the examination of various berry colours a link between the colouration and TPC, FRAP could be sorted out. Since the extraction was carried out using MeOH:water:HCOOH therefore the effect of carotenoids on the free radical scavenging was not measured. Although these compounds also greatly contribute to the overall antioxidant capacity, peppers contain numerous other antioxidants such as ascorbic acid, capsaicin and polyphenols. All the given data are expressed on dry weight basis (dw), the moisture content of the plants at different phenophases were around 91% and not significantly different from one another.

Phenophase and genotype effect on TPC and on FRAP values

The results of TPC when expressed on dw in the tested genotypes ranged between 35 mmol gallic acid equivalent/g in genotype 6 and 88 mmol gallic acid equivalent/g in genotype 1 at green stage 1. At green stage 2 however, a 4-fold variation was observed between genotype 1 and 3. Even higher differences were detected at the turning phase between the same genotypes and these proportions remained the same at ripe stages as well (Fig. 2).

Except for genotype 1 and 6, all peppers showed higher TPC values at both green stages than in their fully ripe phase, which is in line with Deepa et al. (2007) study [10]. Numerous reasons could account for these elevated values i.e.: plant tissues used, genotype effect, biotic or abiotic stresses etc. However, in case of genotype 2, 3, and 4 the additive effect of the anthocyanins in the unripe berries could also cause higher TPC values. As of genotype 1, although it has a purple berry at its unripe stages, it remains purple throughout ripening building anthocyanins up until its turning phase. Sample 1 was the single genotype with significantly higher TPC levels at nearly all stages of maturity. However, sample 7 also exhibited high values at green stage 1, 84.57 mmol gallic acid equivalent/g, while the extreme lilac sample 1 had 88.74 mmol gallic acid/g. An overall decreasing trend is visible from the TPC values during ripening which is in correlation with Marin et al. (2004) findings [11].

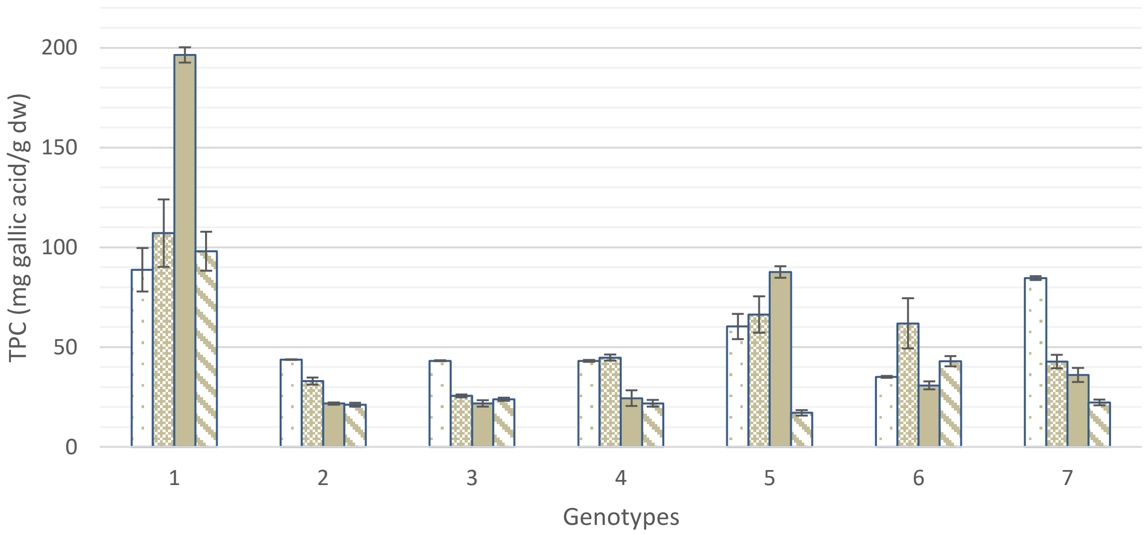

Fig. 2 TPC of seven mutant Capsicum genotypes in four stages of ripening. Green stage 1: ☐ , Green stage 2: ☐, Turning phase: ☐ , Biological ripe phase: ☐

As of their antioxidant capacity, it was measured against their iron reducing capacity by the FRAP assay. A similar trend was observed during maturity as in the case of TPC which is supported by a line of researches. All genotypes – except for sample 1 – showed high values of antioxidant capacity at green stage 1, then a decline phase at their intermediate stages, followed by a slight increase at biological ripeness (Fig. 3). Genotype 1, in addition to containing high levels of anthocyanins throughout maturation, it also contains compounds responsible for the pungency of the berry, therefore the capsaicin could also add to the overall higher values of antioxidant capacity. At their ripe phases there is a 5.5-fold difference between the extreme lilac (genotype 1) and the plant that never turns purple (genotype 7).

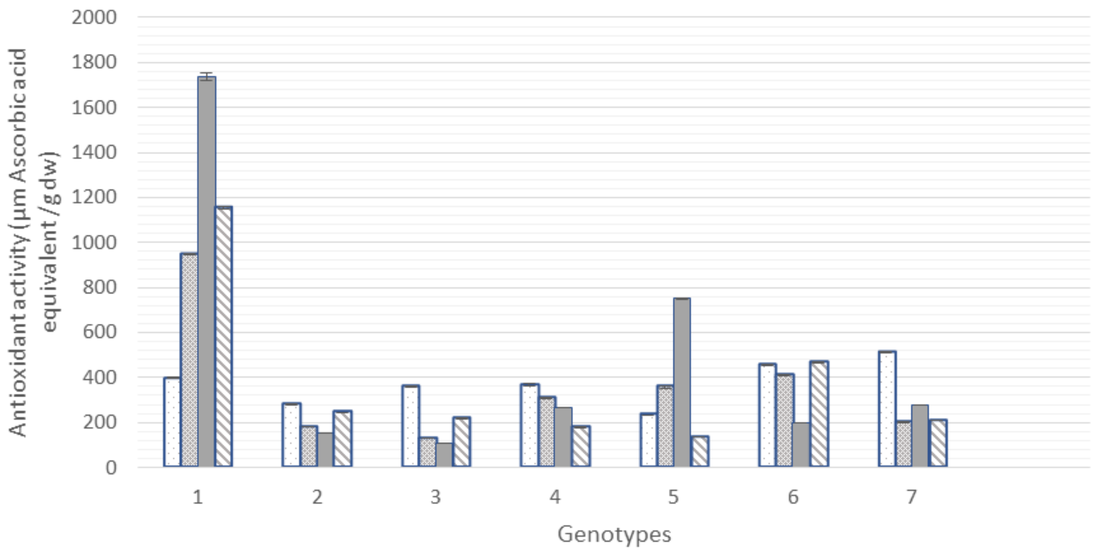

Fig. 3 Antioxidant power of seven mutant Capsicum genotypes in four stages of ripening. Green stage 1: ☐ , Green stage 2: ▩, Turning phase: ■ , Biological ripe phase: ▧

Both the genotype and the ripening stage had a significant effect on both the TPC and on the FRAP value. The correlation between the phenolic content and scavenging free radical activity of the peppers when expressed on dry weight basis were significant in every stage examined, except for the green stage 1 where only 9% of the antioxidant activity is explained by the phenolic content. Numerous studies arrived at the same conclusion that TPC and FRAP values are closely correlated, our results show R^2=0.89 at green stage 2, R^2=0.99 at turning just like at the ripe stage [12, 13].

Conclusion

Significant variations in both TPC and FRAP values in between genotypes and phenophases indicate that the nutritional composition of the berries changes during maturation in different genotypes. The extreme lilac sample (genotype 1) exhibited outstanding results for both TPC and FRAP, indicating that the presence of anthocyanins could lead to a rich dietary composition. Although studies indicate that the ripe – yellow, orange or red coloured – berries exhibit higher values for both assays, our hypothesis that the anthocyanin build-up at the early stages of the ripening together with the genotype effect would lead to higher values in the green stages appears accurate [12].

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