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Effects of Long-term Nitrogen and Organic Fertilization on Antioxidants Content of Tomato Fruits

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Abstract

The effects of different N level in combination with organic fertilizer on carotenoids, phenols and flavonoids contents in tomato fruits were studied. The N mixed with organic fertilizer treatment had higher the content of β-carotene, and AN2 achieved 34.20 μg/g. At red ripening stage, the content of lycopene of BN1 and BN2 were very close to, respectively 180.79 μg/g and 182.50 μg/g. The content of lutein at red ripening stage was nearly three times than that at turning stage. At red ripening stage, content of lutein ranged from 2.85 μg/g to 8.87 μg/g. In the all of phenolic acid, coffeic acid was the highest levels. The highest caffeic acid content (73.74 μg/g) was observed in the AN2 (double N and organic fertilizer), and only organic fertilizer (AN0) was no significant difference with BN1 (single N). Rutin content in tomato fruit had no difference in three N levels (N0, N1, N2). AN0 had the highest quercetin content in tomato fruits in all treatment, by 66.39 μg/ g.

Keywords: Tomato; Carotenoids; Phenolic acid; Flavonoids; Fertilization

Introduction

Tomatoes are planted in worldwide because its fruits have many nutrients and high yields. As an important vegetable, they are an excellent source of vitamin C, vitamin E, folic acid, potassium, and antioxidants [1-3]. Since the early 1970s, antioxidants content in tomato fruits became a focus. Main antioxidants in tomato fruits include carotenoids, phenols and flavonoids. Immune system of body can reduce the incidence of cancers and other diseases by using carotenoids to adjust [4,5]. Meanwhile lycopene is providing protection against various cancers, angiocardiopathy, and other chronic diseases, probably due to its antioxidative properties [6]. Phenolic acids have stronger ability to resistant oxidation and damage of normal cells when it accumulates in body [7,8]. It can efficiently wipe out the singlet oxygen and peroxide free radicals of body. Flavonoids also can efficiently reduce radical which make the person aging to delay aging, because they have strong antioxidant activity.

Biosynthesis of lycopene and other carotenoids in plants comes from mevalonic acid and AcCoA. Plant phenol compounds are mainly through shikimic acid pathway and malonic acid pathway to synthetic. Flavonoids are the product of shikimic acid and more ketone ways, and biosynthesis of flavonoid is the basic skeleton of the three Malonyl CoA and 1 coumaric acyl coenzyme A (coumaroyl CoA). Those all are closely related to the nitrogen which is components of enzymes and plays an important role in metabolism and energy conversion [9-11]. So the antioxidant capability in tomato fruits is strongly affected by fertilization. But information on antioxidant contents of tomato affected by fertilization is scarce. Aldrich et al. and Anissa Riahi et al. focused on the total phenols content and lycopene content affected by different cultivars under fertilization. In addition, our research results showed that N combination with organic fertilizer can promote tomato yield and quality of tomato. However, in that study, antioxidant content of tomato fruits was not determined. Now the determination of antioxidant content in tomato fruits allows a real evaluation of nutritional quality of food rather than the analysis of basic sugar, protein, and basic content. In fact, these antioxidant contents contribute to the health benefits of body which people care about it [12-14].

In the last decade of the last century the excess of N becomes widespread in agriculture, namely as a consequence to pursuit of high yield and also led to the decrease of the quality of crops [15,16]. The objective of this study was to investigate response to different levels N fertilizers in combination with organic fertilizers on carotenoids, phenols and flavonoids contents in tomatoes fruits.

Materials and Methods

Materials and treatments

Tomato (liaoyuanduoli) designed for field growing and test was examined during the year 2014 growing season (March-July). Tomato seedlings were grown under constant conditions of temperature 25°C/20°C (day/night), photoperiod 16 h light, 8h dark, illumination 250 μE/s/m. After 50 days the plants were transplanted to the plots in greenhouse.

The trial was done at the vegetable research station of Shenyang Agricultural University (Loc: 41°20'N, 123°38'E), China. The long-term fertilization experiment began in 1988, and the first 8.5 years proceeded in the open with a year of two stubbles and moved to an enclosed facility with a year of stubble in 1997. The fertility of foundation soil before the experiment was such that organic matter content was 24.3 g/kg, total N 1.164 g/kg, total P 1.374 g/kg, available N 86.41 g/kg, available P 70.8 g/kg, available K 56.14 g.kg and pH 6.75. This trial used to denote plots manure (AN0), manure +N fertilizer (AN1), manure +double N fertilizer (AN2) without fertilization (BN0), N fertilizer (BN1), double N fertilizer (BN2). A completely randomized design, replicated three times was selected with plot sizes of 1.5 m^2. To prevent fertilizers permeating into adjacent plots, each plot was built as a 0.8 m deep sealed cement pool. Manure was applied once before planting while N fertilizer was applied twice during the growing season. The experiment was finished after 4 month and half of plants growing.

Tomato fruits were hand harvested randomly from the rows and from the middle of each plant at three stages (turning, red, red ripening) and delivered quickly to the laboratory. Each tomato sample (whole fruit) washed, and then cut into small pieces and freeze-dried under an inert nitrogen atmosphere at -70°C. The freeze-dried samples were ground in a coffee grinder and screened through a size-20 standard sieve to obtain uniform particle size, then stored in deeper freezer until analyzed.

Treatment	Horse manure(g/plot)	Urea (g/plot)
AN0	11.25	0
AN1	11.25	97.8
AN2	11.25	195.6
BN0	0	0
BN1	0	97.8
BN2	0	195.6

Table1: Treatment of experiment.

Chemical reagents

Standards (lycopene, β-carotene and lutein), objects,and BHT were purchased from Shanghai Yuanye technological company. Phenolic acids and standards (caffeic acid, ferulic acid, cinnamon acid, chlorogenic acid, benzoic acid, anthocyanin, rutin, quercetin, luteolin, kaempferol) were purchased from Sigma. All of HPLC-grade solvents were purchased from Fisher Chemicals.

Analytical procedures

Determination of carotenoid: 0.2 g homogenate was used for carotenoid extraction. The mixture of acetone: petroleum ether (2:1) was used for carotenoid extraction, adding 10 μg internal standard substance. The vials were then placed in a sonicator bath at 30°C for 30 min. The mixture was centrifuged and the supernatant was transferred into a 10 mL tube. After vacuum freeze-dried of the supernatant, using 1.0 mL ethyl acetate dissolved and then filter the 0.45 μm micro porous filter membrane. The filter was used for HPLC analysis [17].

Column: Nova-pak C18, 4 μm, 3.9 mm × 150 mm; temperature: 30°C, mobile phase: acetonitrile: trichloromethane (92:8), flow rate:1.0 ml per minute, sample loop 20 μl, spectrophotometric detector λ=450 nm. Standard of lycopene, β-carotene and lutein was used for peak identification and quantification.

Determination of phenol: For each extraction, approximately 0.2 g of ground freeze-dried tomato sample was placed in a 20 ml tube with 5 mL of the solvent mixture ethanol:H_2O (50:50,%v/v). The vials were then placed in a sonicator bath at 60°C for 20 min.The mixture was centrifuged and the supernant was transferred into a 10 mL tube. The residue was resuspended in 5 mL of mixture (above) and mixed, then sonicated for an additional 20 min followed by centrifugantion. The supernant was combined with the initial extract and the volume of combined wupernant was made up to 10 mL with the extraction solvent and waited for HPLC [18].

Phenolic acids were analyzed on a HPLC. Separation of phenolic acids was achieved on a reversed phase C18 Luna column (Phenomenex, 3.9 mm × 150 mm; particle size 4 μm). The column was thermostatically controlled at 30°C and the flow rate was set to 1.0 mL/min. The mobile phase consisted of two solvents: 3% (ethylic acid (A) and methanol (B). Two wavelengths (280 nm and 320 nm) were used to detect the eluent composition. All phenolic acids were quantified with external standards by using HPLC analysis.

Determination of flavonoid

For each extraction, approximately 0.2 g of ground freeze-dried tomato sample was placed in a 20 ml tube with 5 mL of the solvent mixture ethanol: H_2O (50:50,%v/v). The vials were then placed in a sonicator bath at 60° for 20 min.The mixture was centrifuged and the supernant was transferred into a 10 mL tube. The residue was resuspended in 5 mL of mixture (above) and mixed, then sonicated for an additional 20 min followed by centrifugation. The supernatant was combined with the initial extract and the volume of combined supernatant was made up to 10 mL with the extraction solvent and waited for HPLC.

Column: Nova-pak C18, 4 μm, 3.9 mm × 150 mm; temperature: 35, mobile phase: 0.5% phosphoric acid:methanol:(1:1), flow rate:1.0 ml per minute, sample loop 10 μl, spectrophotometric detector λ=360 nm.

Statistical analysis

Effect of fertilizer treatments on antioxidant in tomato fruits were assessed by analysis of variance (ANOVA) and comparison among means was determined according to Tukey's test.

Results

Carotenoids content

Influence of fertilization on β-carotene content is given in Figure 1. Compared to the single N treatment, the N mixed with manure treatment had higher the content of β-carotene, among of those, AN2 achieved 34.20 μg/g. Followed the tomato fruits mature, the content of β-carotene was dramatically increased. Different level N showed different content of β-carotene. In all, the more N number, the higher content of β-carotene.

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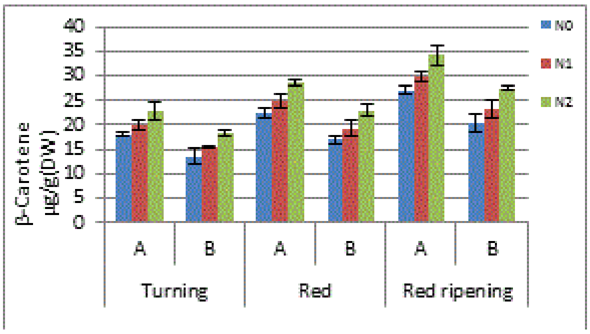

Figure1: Carotenoids content in tomato fruits under different N levels mixed with organic fertilizer.

Influence of fertilization on lycopene content is given in Figure 2. Followed the tomato fruits mature, the content of lycopene was increased. There was no significantly different content of lycopene between the red and red ripening stage of fruits, but they were all higher than the turning stage. N with manure increased the content of lycopene compare with single N, and the more N number, the higher content of lycopene. But at red ripening stage, the content of lycopene of BN1 and BN2 were very close to, respectively 180.79 μg/g and 182.50 μg/g that was to say over N level had nothing to do with lycopene content. But N mixed with organic, higher N level increased lycopene content, so AN2 had the highest content of lycopene, 238.42 μg/g.

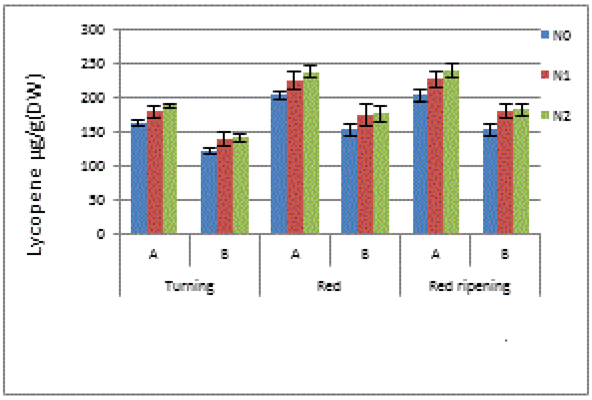

Figure 2: Lycopene content in tomato fruits under different N levels mixed with organic fertilizer.

Influence of fertilization on content of lutein is given in Figure 3. Followed the tomato fruits mature, the content of lycopene was increased. The content of lutein at red ripening stage was nearly three times than that at turning stage. N fertilizer and mixed with manure could increase the content of lutein compared to only N fertilizer. At red ripening stage, content of lutein ranged from 2.85 μg/g to 8.87 μg/g, AN0 to AN2. The content of lutein of only manure (BN0) was higher than that of sing N treatment (AN1), and had on difference with the double N fertilizer (AN2).

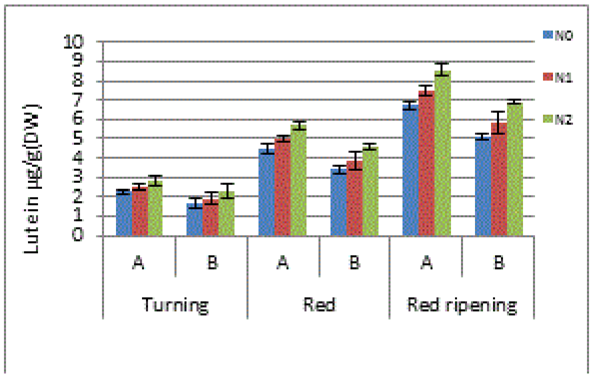

Figure 3: Lutein content in tomato fruits under different N levels mixed with organic fertilizer.

Phenolic acid content

Phenolic acids and total phenol contents of tomato fruits grown under the different fertilizer treatments are shown in Figures 4 and 5, respectively. The contents of caffeic acid, cinnamon acid, ferulic acid, chlorogenic acid, benzoic acid and anthocyanin in tomato varied depending on the different N level and mix with organic fertilizer. In general, all these phenolic compounds increased after N mixed with organic fertilizer, and improved with N level increased. In the all of phenol acid, coffeic acid is the highest levels. The highest caffeic acid content (73.74 μg/g) was observed in the AN2 (double N and manure), and only organic fertilizer AN0 was no significant difference with BN1 (single N). Benzoic acid and anthocyanin were the lowest levels in phenolic acid.

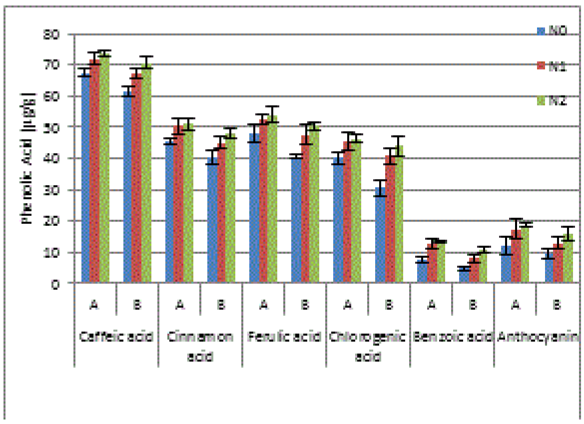

Figure 4: Phenolic acids content in tomato fruits under different N levels mixed with organic fertilizer.

Total phenol contents of AN1 and AN2 had no difference, but higher than that BN2, 250.7 μg/g, 257.12 μg/g respectively.

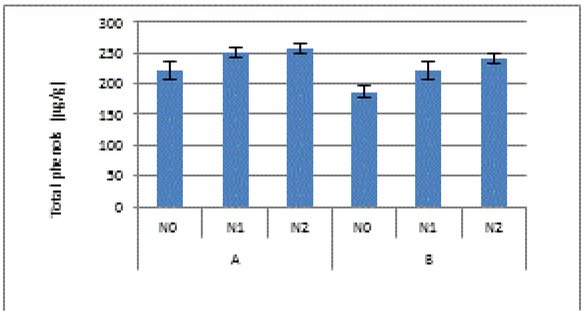

Figure 5: Total phenos content in tomato fruits under different N levels mixed with organic fertilizer.

Flavonoid content

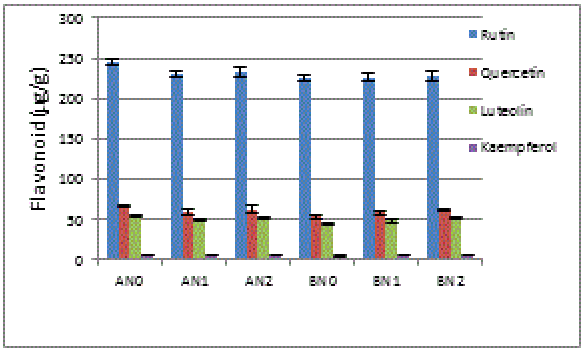

Figure 6: Flavonoid content in tomato fruits under different N levels mixed with organic fertilizer.

Flavonoids detected in tomato including rutin, quercetin, and Luteolin, kaempferol, among them the highest content of rutin, the lowest content of kaempferol. Different nitrogen levels had different effects on content of flavonoids. Organic with N fertilizer increased different flavonoids contents, higher than only N ferlilizer. So, organic fertilizer had significant effects on flavonoid content. Rutin content of fruit of tomato had on difference in three N levels (N0, N1, N2). AN0 had the highest quercetin content of tomato fruits in all treatment, by 66.39 μg/g, but the BN0 had the lowest content, only 53.65 μg/g. Under only N fertilizer, with the increase level N, the content of quercetin, luteolin,and kaempferol increased as well.

Discussion

In the different stage of fruit, β-carotene content in tomato fruit showed a rising trend, and at turning stage β-carotene content in the fruit was low just because its coloring just began to form. But β-carotene content increased rapidly at red stage, mainly due to β-carotene fully formed [19,20]. The results of this study showed that fully mature tomatoes under different N levels forms the accumulation of carotenoids, its main content was lycopene, about 80% of the total carotenoid content, followed by β-carotene content of only about 20% of the total carotenoid content, lutein content at least. But this study we used only one tomato varieties, carotenoid content in different colors, different varieties may vary, so the mechanism needs further to study. The results showed that from turning to red ripening, the development of the lycopene content in tomato fruit changed significantly, and that synthetic lycopene, are the fruits of lycopene synthesis in critical period, this is consistent with most of the scholars ' research [21,22]. Letein content under different N levels in the whole growth period was on the rise, and N mixed with organic fertilizer had greater effects than the inorganic fertilizer treatment.

Plants rich in many types of phenolic compounds and its structure are different too [23]. Studies have shown that with the increase of fruit maturity increased phenolic compounds in fruits; this is consistent with the conclusions of this experiment [24]. Studies have shown that different growth conditions and different temperature light will lead to the different content of phenols and different constitute [25]. External conditions on the content of phenolic compounds in plants also have an impact [26]. This experiment selected common phenolic compounds in tomato fruit were detected and found phenolic compounds include caffeic acid, chlorogenic acid, ferulic acid and cinnamic acid, benzoic acid and anthocyanin. As the test for liquid chromatography and found the presence of unknown peak, so tomato fruit may also have other types of phenolic compounds in existence, their specific situations to be in later generations studied. Different from the results of this test, this may be associated with years of fertilization. Effects of different nitrogen levels on phenolic compounds in fruit accumulation and synthesis, specifically nitrogen can promote the synthesis of phenolic compounds. Applying organic fertilizer, most phenols content showed a facilitating role, but little effect on the acid and anthocyanin contents. Different levels of nitrogen fertilizer and organic fertilizer can increase the content of phenols, and twice times the nitrogen fertilizer and organic fertilizer under the condition of phenolic acids content in tomato fruits reaches its maximum value.

Flavonoids of synthesis and accumulation are a complex physiological and biochemical processes, this process requires many different genes to control [27]. Process of synthesis and accumulation of flavonoids, enzymes play a crucial role [28]. These enzymes include: flavanone reductase, isoflavone syntheses, chalcone reductase, etc., The activity of these enzymes will affect total flavonoids from fruits of synthesis and accumulation. We have previously found that the flavonoid content of tomato varies duting p production season dut to both abiotic (e.g., light) and agronomic (e.g., fertilization) factors [29]. Different minerals can change solutions of phenylalanine ammonia-lyase activity. Studies have shown that with the increase of fruit maturity flavonoids in fruits increased, consistent with the conclusions of this experiment. Studies confirm that a moderate reduction of application of inorganic fertilizer can increase plant flavonoids accumulation and synthesis. In N-fertilizer treatment, the flavonoids content in tomato fruits under N2 treatment had higher than that of N1 level, specifically N2>N1>N0. Mixture of organic fertilizer had significant effects on flavonoid content; diagrams can be found that combined application organic fertilizer can increase the content of flavonoids in fruit.

Conclusion

This current study highlights the difference in antioxidant content including carotenoids, phenols, and flavonoids contents of tomato grown in different N level and different fertilizer combinations. The results indicated higher level N had a higher level of carotenoids, in particular the β-carotene. Organic fertilizer can promote the phenols and flavonoids content, especially mixed with N. So we can mention that we should use organic fertilizer mixed with chemical fertilizer other than only chemical fertilizer, which not only improve tomato quality, but also build soil fertility.

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The authors declare that they have no conflict of interest.

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