Effects of Some Nutrition Experiments on Ellagic Acid end Nitrate Contents in Fruit in Organic Strawberry Production

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Key words: Organic strawberry, ellagic acid, nitrate

Abstract

In this study carried out in Isparta province of Turkey in 2005 and 2006, it was determined how ellagic acid and nitrate contents of fruit were affected from some application performed in organic and conventional strawberry production. In the study, 18 different application were made at Camarosa strawberry cultivar. The plants were planted as frigo seedlings in the third week of July and irrigated by drip irrigation method. According to the results; ellagic acid contents were ranged from 0.487 to 0.498 mg 100g^{-1} and there were no significant differences among values. Nitrate contens were ranged from 1.43 to 4.57 mg kg^{-1} and there were significant differences among values.

Introduction

Organic agriculture is an alternative method in the settlement of environmental and health problems resulting from the gradually intensifying use of agricultural inputs (Aksoy, 2001). As organic agriculture has gradually become widespread, the production of plant growth regulators and nutrient preparations which might be used in organic growing has begun to multiply. The total strawberry production of Turkey is about 300 thousand tons (Anonymous, 2013a), and of this production, approximately 4 thousand tons are produced under organic conditions (Anoymous, 2013b). The organic strawberry production is rapidly increasing in Turkey as all around the world. This fruit is particularly rich in vitamin C, and 100 g of this fruit contain up to 100 mg of vitamin C (Türemiş et al., 2000). It is reported that the amount of fructose in strawberry ranges from 5.40 to 11.00 g 100ml^{-1} and its amount of organic acid from 1.20 to 1.80 g 100 ml^{-1} according to cultivars (Hakala et al., 2002). Ellagic acid is an anticarcinogenic and antimutagenic phenolic compound which is important to cardiovascular diseases. Ellagic acid is the one which is found at the highest amount in terms of phenolic compounds in strawberry (Koşar et al., 2004; Hakkinen, 2000). Strawberry contains a higher amount of ellagic acid than many fruits Williner et al. (2003), and the amounts of ellagic acid in fruits vary by cultivar and harvest maturity (Maas et al., 1991). An important issue to which attention must be paid in fruit & vegetable growing is that the nitrate and nitric contents of the edible parts should be within the limits which will not be deleterious to human health. This issue becomes far more important especially in organic fruit growing. The researchers reported that nitrates and nitrites were present naturally in vegetables, fruits, forage crops, and freshwater and that the extra nitrates and nitrites in foods directly threatened human and animal health (Maas et al., 1991; Osweiler, 1985).

Material and methods

The study was conducted in Eğirdir (Isparta) in 2005 and 2006. The plants were planted in the form of a square of 30 cm X 30 cm on the beds which were 30 cm in

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height and 100 cm in width. Planting was performed with frigo seedlings in July, and the experiment was set up biennially according to the randomized block experimental design with 5 replications. The Camarosa cultivar was used as the plant material. Irrigation was performed in the form of drip irrigation, and weed control was carried out by using a hand hoe and mulch (black polyethylene).
The applications whose effect was tested in the study are as follows: 1-Organic growing (15 different nutrient applications and the C-1 application with no nutrient application); 2-Conventional growing (CG); and 3-Control (C-2). Farm manure (FM), green manure (GM), clinoptilolite (Cln), seaweed (S) and their combinations were tested as nutrient applications in organic growing, while nitrogen had been applied before planting, and nitrogen and phosphorus were applied in the first year and nitrogen, phosphorus and potassium in the second year according to the results of the soil analysis in conventional growing. In addition, iron was applied to both all applications in the organic plot and the conventional plot in the second year. However, no nutrient or agricultural control was applied in the control. Statistical analyses were performed using analysis of variance in JMP statistical software. The means were separated by Tukey.

To determine the ellagic acid content, the fruits were frozen in liquid nitrogen following the harvest and left in a deep freezer till the analysis. 3 g of fruits were obtained for ellagic acid extraction and mixed with 10 ml of acetone:water (at the ratio of 1:4); 0.1 ml of TFA (Trifluoressiqsaure acetic acid) was added; and they were extracted for an hour in the 100^{o}C water bath. The specimens were filtered and read at 200-600 nm wavelengths in liquid chromatographic apparatus (Shimadzu WP HPLC), and the obtained results were detected in mg 100g^{-1}. The detector used was Photodiode Array detector; the column was Nucleosil C 18 (150mm x 4.6mm); and its flow rate was 1 ml/minute. Solvent A was prepared by mixing 2.5% formic acid (HCOOH) in water, while Solvent B was prepared by mixing 2.5% formic acid in acetonitrile (Koşar et al., 2004).

To determine the nitrate content, first of all it was intended to determine the amount of nitrite in 500g of fruits obtained randomly; however, no nitrite could be detected in any of the specimens, and it took the value of zero (“0”) in the formula. For nitrite detection, the sample was extracted with hot water (40-45^{o}C), and the proteins were precipitated with potassium hexacyanoferrate and zinc acetate and filtered. A red complex was created by adding sulphanilamide chloride and N-(1-Naphthyl) Ethylenediamine Dihydrochloride to the filtrate, and it was read at 538nm wavelength of Biocrom 8500 II spectrophotometer. For nitrate detection, the filtrate in the nitrite detection was reduced to nitrite with metallic cadmium; a red complex was created using the resulting filtrate and the color reagent; and it was read at 538nm wavelength by a Biocrom 8500 II spectrophotometer and computed with the following formula (Anonymous, 2000). NO_3 (ppm) = 1.348 x [(Dilution rate x the value read in the spectrophotometer)-(NO_2 conc.)]

Research and Discussion

Ellagic Acid: The values of ellagic acid are shown in Table 1. When these values were examined, the statistical difference between the applications and years were found insignificant. It was seen that the amount of ellagic acid in Camarosa strawberry cultivar did not vary by application and that the values ranged from 0.487 mg 100g^{-1} to

0.498 mg 100g^{-1}. The researchers detected 0.36 mg 100g^{-1} of ellagic acid in the ripe fruits of strawberry (Koşar et al., 2004). In another study, it was stated that no difference in the total amount of phenolic acid (ellagic, p-coumaric, caffeic, and ferulic acids) was seen between the fruits obtained from organic and conventional growing (Hakkinen, 2000). These data overlap our findings.

Nitrate: The amounts of nitrate in the fruit are demonstrated in Table 1. When these values were examined, the statistical difference between the applications and years were found significant. According to the data in the experiment, the highest values were obtained from conventional growing in the first year (4.57 mg kg^{-1}) and in the second year (4.06 mg kg^{-1}), whereas the lowest values were obtained from S (1.68 mg kg^{-1}) in the first year and from C-1 (1.43 mg kg^{-1}) in the second year. According to the amounts of nitrate it contains, the strawberry fruit is included in the moderate-nitrate group (200-600 mg kg^{-1}) (Anonymous, 2000). When the data obtained from the experiment were evaluated, it was seen that these limits were not reached in any of the applications (0-200 mg kg^{-1}).

Table 1. Ellagic acid values and Nitrate values

Applications	Ellagic acid values (mg 100g^{-1})			Nitrate values (mg kg^{-1})		
	2005	2006	Average	2005	2006	Average*
FM	0.492	0.491	0.492	3.60	2.03	2.82 ^{bc}
GM	0.492	0.489	0.491	1.98	1.68	1.83 $^{d-f}$
Cln	0.490	0.488	0.489	2.17	1.62	1.90 $^{c-f}$
S	0.494	0.494	0.494	1.68	1.73	1.70 ^{ef}
FM+GM	0.493	0.493	0.493	2.91	2.47	2.69 $^{b-d}$
FM+Cln	0.494	0.496	0.495	3.86	1.92	2.89 ^{b}
FM+S	0.495	0.493	0.494	3.08	2.03	2.53 $^{b-f}$
FM+GM+Cln	0.495	0.489	0.492	2.79	2.57	2.68 $^{b-d}$
FM+GM+S	0.494	0.490	0.492	2.97	1.96	2.46 $^{b-f}$
FM+GM+Cln+S	0.494	0.491	0.492	3.09	1.87	2.48 $^{b-f}$
FM+Cln+S	0.489	0.497	0.493	2.57	2.17	2.37 $^{b-f}$
GM+Cln	0.496	0.497	0.497	2.42	2.21	2.32 $^{b-f}$
GM+S	0.496	0.487	0.491	2.38	1.87	2.13 $^{b-f}$
GM+Cln+S	0.493	0.496	0.495	2.55	2.21	2.38 $^{b-f}$
Cln+S	0.490	0.498	0.494	2.84	2.40	2.62 $^{b-e}$
C-1	0.494	0.494	0.494	1.90	1.43	1.67 ^{f}
CG	0.491	0.491	0.491	4.57	4.06	4.31 ^{a}
C-2	0.490	0.492	0.491	1.88	1.81	1.85 $^{d-f}$
Average *	0.492	0.492		2.70 ^{a}	2.14 ^{b}	

* significant at $P<0.05$

Conclusion

When the applications in organic strawberry growing and conventional strawberry growing are compared in terms of the values of ellagic acid, it is seen that the

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difference between them is statistically insignificant. According to the averages of both years, the values of ellagic acid from 0.489 to 0.495 mg $100g^{-1}$. It is known that an organically grown strawberry is healthier than those which are conventionally grown. Nevertheless, the fact that nitrate and some metals are higher than the specified limits threatens human health and might cause the formation of diseases up to cancer. According to the values of nitrate obtained, it was detected that the values obtained from conventional growing both in the first year (4.57 mg kg^{-1}) and the second year (4.06 mg kg^{-1}) were higher than the values in organic growing (1.43-3.60 mg kg^{-1}). Although the strawberry fruit was stated in the moderate-nitrate group (Anonymous, 2000), the values obtained from all applications in the experiment were included in the low-nitrate group. According to these findings, it might be stated that all products obtained from both organic and conventional growing are safe in terms of nitrate.

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