Phosphorus Fertilization and Biosynthesis of Functional Food Ingredients

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Abstract

The disease-preventive and health-restorative effects of fruit and vegetable consumption have been related to the action of several nutraceutical components, positively affecting the physiological functions in humans. Two classes of the major nutraceutical components studied in fruits include flavonoids and isoprenoids. Flavonoids such as quercetin and catechin are strong antioxidants and powerful inhibitors of calcium (Ca) second messenger function. Isoprenoids such as lycopene and carotene are also strong antioxidants. In general, these components are believed to be the principal agents in fruits, vegetables, and their processed products that impart anticancer properties and cardiovascular protection in humans. Phenolic components from fruit wines acted as strong superoxide and hydroxyl radical scavengers. As well, phenolic components from red grape wine inhibited the proliferation of breast cancer cell lines. Therefore, enriching the fruits and vegetables with nutraceutical components could have beneficial effects to the consumers. Both the flavonoid biosynthetic pathway and the isoprenoid pathway are heavily dependent on phosphorus (P)-containing metabolites such as ATP, NADPH, and sugar phosphates derived through the pentose P pathway. Thus, P fertilization may have a direct effect on the levels of these metabolites, and potentially in the levels of the end products such as flavonoids and lycopene. To examine this possibility, we have subjected tomato plants and apple trees to supplemented soil and foliar P (superphosphate, Hydrophos, Seniphos) fertilization. Evidence gathered so far suggests that increased P fertilization can enhance the anthocyanin levels in apples and lycopene levels in tomatoes. Further studies are in progress.

Introduction

Functional food ingredients (nutraceuticals) in fruits and vegetables such as flavonoids, terpenes, lycopene, etc. are biosynthesized through pathways that use reducing power in the form of NADPH, and ATP. As well, the biosynthetic origin of many of the functional food ingredients occurs from the pentose phosphate pathway, which is a key metabolic pathway also involved in the antioxidant defense system. Higher levels of nutraceuticals will increase the nutritional quality of fruits and vegetables since the nutraceutical components have been found to exert beneficial effects on health of the consumers. The effect of P fertilization on the levels of functional food ingredients has not been investigated. To our knowledge, this is the first systematic study into the physiology and biochemistry of nutraceutical biosynthesis. In this study, we have used two varieties of apples (Red Delicious and McIntosh), and several varieties of tomatoes to evaluate the effects of P fertilization. Phosphorus fertilization was provided either as superphosphate or as foliar spray (Hydrophos and Seniphos; Phosyn plc, UK).

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Apples

Objective

To evaluate the effect of soil and foliar P supplementation on the post harvest quality of apples (*Malus domestica* Borkh. cv. 'McIntosh' and cv. 'Red Delicious').

Methods

Five P treatments were applied to 20 trees (4 reps): control (no P), superphosphate (soil applied, 0-20-0) at a low dose (420g/tree) and a high dose (420g x 3/tree, in 20 dry intervals), and foliar applications of Hydrophos (8g P, 1.2g magnesium (Mg) in 4L) and Seniphos (11.6g P, 1.5g Ca in 4L).

For foliar treatments, one side of the tree was sprayed throughout the season at four 20-day intervals. Superphosphate treatments were applied to the entire tree, but apples were still separated into 'sprayed' and 'non-sprayed' sides to reduce other field effects. Apples were harvested at optimal maturity and stored in air at 0°C for 4 months.

Results

Phosphorus fertilization did not affect the fruit weight, firmness, starch, and internal ethylene of either 'McIntosh' or 'Red Delicious' fruit at harvest (data not shown). The soluble solids content of 'McIntosh' fruit was increased slightly by superphosphate and Hydrophos (**Figure 1**).

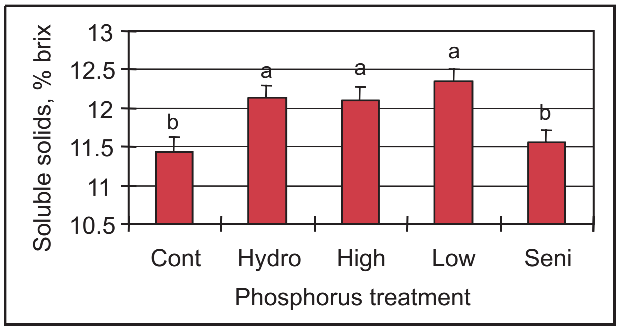

Figure 1. Mean (n=20) and standard error of soluble solids content of 'McIntosh' at harvest. Values with the same letter are not different (p<0.05);

Phosphorus fertilization increased the percentage of red skin on both varieties at harvest (**Figures 2 and 3**) as well as the intensity of the red color in Red Delicious fruit at harvest (**Figure 4**). Fruit from sprayed sides of the trees with foliar treatments had more red color than those from the non-sprayed side (data not shown).

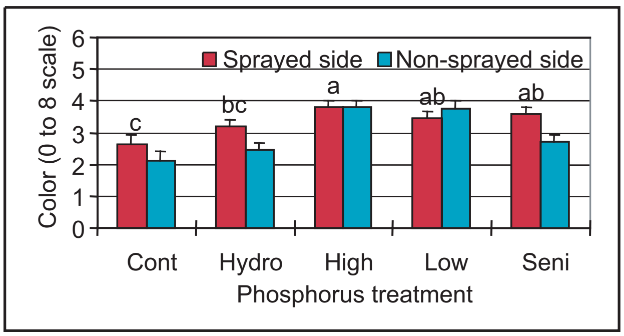

Figure 2. Color of 'McIntosh' at harvest (higher values indicate more red color). Letters compare 'sprayed' sides of treated trees with 'sprayed' and 'non-sprayed' sides of controls. Values with the same letter are not different (p<0.05); mean (n=15) + S.E.

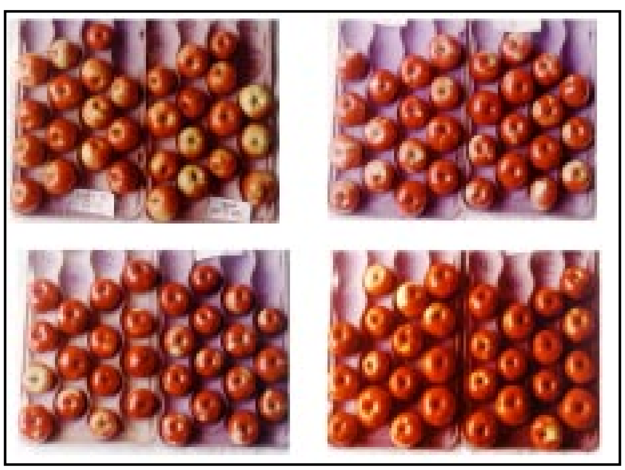

Figure 3. 'Red Delicious' apples at harvest.

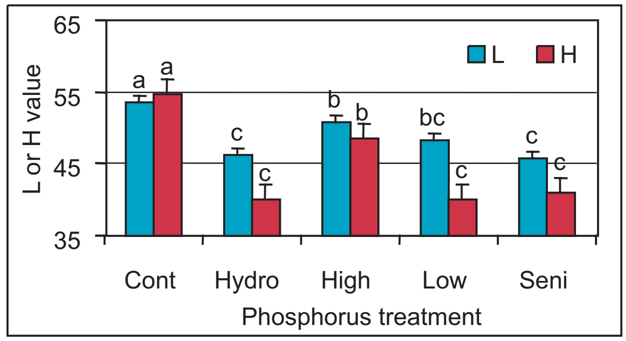

Figure 4. Evaluation of red color of 'Red Delicious' at harvest by colorimeter. Lower L-values indicate a darker (deeper red) color and H-values differentiate between red and green, with lower values indicating red. Values with the same letter are not different (p<0.05); mean (n=15) + S.E.

Phosphorus fertilization decreased the incidence of superficial scald in 'McIntosh' **(Figure 5)**, but not 'Delicious' (data not shown) after 4 months of air storage.

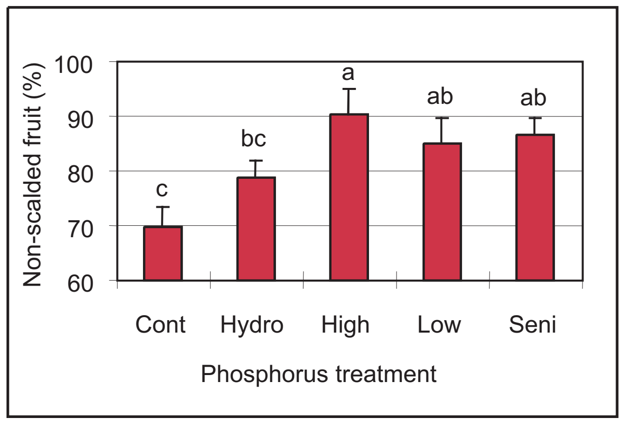

Figure 5. Evaluation of superficial scald on 'McIntosh' after 4 months of air storage plus 7 days at room temperature. Values with the same letter are not different (p<0.05); mean (n=15) + S.E.

Discussion

This experiment was repeated in 2000 with 'McIntosh', but no differences were observed in color or soluble solids at harvest (data not shown). The 1999 season ended with warm sunny days and cool nights—conditions which stimulate anthocyanin production. The 2000 season was more cloudy and not as cool in the nights. After 4 months of air storage some P treatments developed red color (data not shown) to a greater extent, perhaps because of exposure to the cold storage conditions.

Under certain conditions, P fertilization demonstrates some improvements in initial quality and storability; however, a number of environmental factors strongly influence P effects. In particular, day/night temperatures and light exposure influence scald susceptibility and color development.

Tomatoes

The consumption of tomato products is increasing in light of its potential in preventing several forms of cancer, and the antioxidant properties of lycopene, xanthophylls, and lutein.

The objectives of the project were:

1) To evaluate the effect of soil and foliar P supplementation on lycopene biosynthesis, its compartmentation, and levels in tomatoes.
2) To establish the relation between P supplementation and antioxidants, as well as the activities of antioxidant enzymes in tomato.
3) To evaluate the relation between the antioxidant status and the stability of lycopene in processed tomato products.

Materials and Methods

Tomato seeds (*Lycopersicon esculentum* Mill. H9478, H9997, Heinz processing varieties) were germinated in potting soil in the greenhouse. Four-week old plants were transplanted in early June at the Cambridge Research Station of the University of Guelph. Tomato seedling were planted in 1.8 m x 3 m plots, each plot containing 24 plants. Each plot was separated from the others by a minimum distance of 5 ft. Fertilizer applications were based on Ontario Ministry of Agriculture and Food (OMAF) recommendations. The regular P (RP) plots received 50 kg P_2O_5/ha supplied as 5-20-20 and N as 80 g of ammonium nitrate at the time of planting. The low (LP) and high (HP) P-supplemented plots received an additional 120 and 240 kg P_2O_5/ha, respectively, as superphosphate (0-20-0). Two foliar sprays manufactured by Phosyn were applied in separate treatments to plots fertilized at the RP rate: Hydrophos (440 g P_2O_5/L, 74 g K_2O/L, and 60 g/L Mg) at 15 kg P_2O_5/ha, and Seniphos (310 g P_2O_5/L and 40 g/L Ca) at 21 kg P_2O_5/ha. The sprays were applied two times at 15-day intervals during the growing period, diluted in water at 7,400 L/ha. A no P (NP) control was included, which received no P but normal rates of other nutrients. The Olsen soil test P level was 50 mg/L, which is considered high. There were four randomly selected replicates for each treatment.

Hydrophos (Hydro) and Seniphos (Seni) were also applied at a lower dilution (high concentration), which is originally recommended by Phosyn. For the high-concentration treatments, the rate of water application was 200 L/ha. The solutions were sprayed using a hand-held bottle sprayer.

Analysis of Quality Parameters

Physicochemical analysis: The following parameters were analyzed: soluble solids or °Brix, non-soluble solids (NSS - %), ash (%), acidity (%), color Hunter LAB system (L [brightness], a+ [redness], b+ [yellowness], and a/b [color stability] values), lycopene content (mg/100 g of juice), sedimentation stability (precipitate weight ratio [PWR -%], gross viscosity GV [Brookfield viscosity –mPa.s], and serum density [SD –mPa.s]).

Soluble solids (°Brix): The soluble solids content of all samples was measured at room temperature with a hand held refractometer (Fisherbrand, 0 to 25% Fisher Scientific Co.). Readings were expressed as °Brix (AOAC methods 9.32.14 1995). This parameter was measured during processing for all the samples before and after boiling and sterilization.

Total acidity: 10 g of tomato juice was accurately weighed into 250 mL beakers in duplicate. To each sample 200 mL of distilled water was added. The resulting mixture was titrated with 0.1 N NaOH to a pH value of 8.0 in an Accumet Basic AB15 – pH meter (Fisher Scientific Co.). Total acidity was calculated as percentage of citric acid on a fresh weight basis (AOAC Methods 1995; Postlmayr et al., 1956; Nielsen, 1998).

Ash content: 10 g of tomato juice was accurately weighed in pre-dried crucibles. The samples were ashed for 16 hrs. in an Isotemp muffle furnace at 550°C. The ash content was calculated using the formula in AOAC International, 900.02A, 1995.

Phosphorus analysis: A known weight of the fruits was transferred to a crucible, and heated in an oven at 500°C to obtain the ash. The ash was dissolved in 6M sulfuric acid and the phosphate content determined by reacting with molybdate reagent and spectrophotometric estimation.

Non soluble solids (NSS): 10 g of juice and sauce was weighed in pre-dried crucibles. The samples were dry at 105°C for 12 hrs. NSS were measured and calculated according to AOAC Methods 926.08, 1995.

Color: Tomato juice and sauce processed were analyzed using a Minolta CR-300 Chroma meter (Minolta, Ramsey, N.J.) calibrated with a white standard tile. Twenty-five mL of each replicate was transferred to petri plates and the color measurements were performed on the surface of the liquid five times at different places. The chromaticity parameters L, a+, b+, and a/b recorded were the average of five measurements for each replicate (Cheng & Shewfelt, 1988; Baldwin et al., 1991).

Consistency (Brookfield viscosity): Jars containing 250 mL of juice and sauce samples were analyzed using a Brookfield Synchro-lectric viscometer (Brookfield Engineering Laboratories, Stoughton, Massachusetts) with a No. 4 or a No 5 spindle. Positions as well as settings of the viscometer were prepared to obtain precise measurements. Speed of 10-rpm was used. Readings were taken at the 10th cycle (Takada & Nelson, 1983).

Serum viscosity (SV): Fifteen samples of serum were used. The serum was obtained by centrifugation of the juice at 12,800 rpm for 30 min at 4°C followed by filtration of the supernatant through Whatman # 1 filter paper. A water bath (VWR Scientific) was set at 24°C (room temperature). An Ostwald-Cannon-Fenske capillary viscometer (size #50) for transparent liquids was used for this purpose; 10 mL of serum were weighed to determine the density at 24°C; 7 mL were deposited into the viscometer previously submerged partially into the water bath and left for 2 min to equilibrate temperature. Serum was sucked up until the line of measure with a manual vacuum valve and two readings were made for each sample. The flow time was recorded. The kinematic viscosity (í) is expressed in centistokes (cSt or mm^2/s) and calculated as:

ν = Ct, where:

C = calibration constant of the viscometer, cSt/s and

t = flow time, s,

The dynamic viscosity is calculated as follow:

$\eta = \rho\nu$, where:

η = dynamic viscosity, centipoises (cP) or millipascal-second (mPa.s).

ρ = density, g/mL, at the same temperature used for measuring the flow time t, and

ν = kinematic viscosity, cSt (mm^2/s), (Caradec and Nelson, 1985).

Sedimentation analysis: For determining precipitate weight ratio, approximately 40 g of tomato juice prepared through hot break process, as accurately weighed into a 50 mL pre-weighed glass centrifuge tube. The sample was centrifuged at 12,800ppm x g, for 30 min at 4°C. After centrifugation, the supernatant was removed from the precipitate. Samples were replicated two times. The precipitate with the tube was then reweighed accurately and the precipitate weight ratio was calculated by using the equation of Takada & Nelson (1983).

$$PWR\% = \frac{(\text{Precipitate + tube weight}) - (\text{tube weight})}{(\text{Initial sample + tube weight}) - (\text{tube weight})} \times 100$$

where : PWR = Precipitate Weight Ratio

pH: The pH of all samples was measured at room temperature with a pH meter (Fisher Scientific Company).

Lycopene content: 4 g of tomato juice and sauce were precisely weighed into 250 mL brown bottles to exclude light and protect lycopene from degradation (Sadler et al., 1990). One hundred mL hexane:acetone:ethanol (2:1:1 v/v) was added to each vial, stoppered, and agitated for 10 min on a wrist action shaker (Burrel Corp., Pittsburg, PA). This was followed by the addition of 15 mL of water and further shaking for 5 min. The solution separated into distinct, aqueous polar (65 mL) and non-polar (50 mL) layers. The non-polar phase was removed and filtered with 0.45 µm nylon membrane filter (Fisher Scientific). Fifty µm of the filtered aliquot was subjected to HPLC analysis using an Exterra C18 column (Waters 600S) with acetonitrile:methanol (85:15, solvent A) and methanol:hexane (75:25 solvent B). The elution was started with 100% of solvent A and 0% of solvent B at time 0 and ended with 100% of solvent B, and 0% of solvent A, in a linear gradient during a period of ten minutes. The elution of β-carotene and lycopene was monitored at 475 nm. The lycopene used was 95% pure (Sigma Chemical Co.) and showed a retention time of 3.88 min.

Yield was calculated using the total amount of different harvests of tomatoes. Ripe tomatoes from the plot were harvested and weight determined. Statistical analyses were conducted using a SAS program.

Results

As in previous seasons, at early stages of growth, tomato plants in plots supplemented with high P showed an enhanced vigor and showed better filling of the plots. The plants provided with regular P fertilization, low levels of P supplementation, Hydrophos and Seniphos treatments were very similar. At later stages of growth, no differences could be noticed between any of the treatments.

In general, during the 2002 season, the yield was nearly double that obtained during the previous seasons of study. The yield of H9478 was nearly similar in all treatments (**Figure 6**) reaching 200 t/ha on the average. The yield was slightly lower in H9997 with about 150 t/ha on the average. The high P treatment enhanced the yield in H9997 by about 25 t/ha, which is not statistically significant. However, a similar increase has been observed in previous seasons in response to higher soil P supplementation.

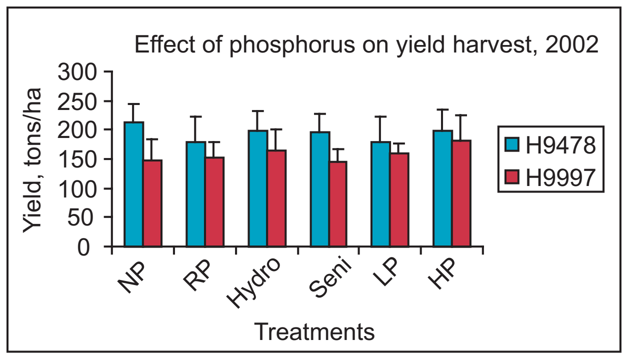

Figure 6. Effect of different P fertilization on the yield of H9478 and H9997 tomatoes.

In all treatments, the yield of H9478 was higher than that of H9997. Within cultivars, there was no significant difference among treatments.

Hydrophos and Seniphos were also applied at a lower dilution (higher concentration), which is usually recommended by Phosyn. Hydrophos application did not have any significant difference on the yield either from NP treatment or regular Hydrophos application (**Table 1**). However, Seniphos application gave an increase in the yield. The increase in yield with low dilution Seniphos application was observed in both H9478 and H9997, which is an interesting observation.

Various physico-chemical parameters of tomato juice were analyzed (**Table 2**) before heat sterilization. The P content of the tomato increased in response to P supplementation from 103 mg in no P to 108 mg in regular P treatment and above 110 mg in low and high soil P supplementation treatments. The Brix level, acidity, and non-soluble solids are very similar in all treatments. The ratio of red (a) to yellow (b) color increased slightly (not significant) in response to Hydrophos and Seniphos treatment. The ash content also increased in low and high soil P supplementation treatments.

The above parameters were also analyzed in H9997 tomato juice preparation (**Table 3**). The results do not show any significant differences between treatments. The P content was higher under low and high soil P supplementation (LP, HP), as seen in the case with H9478.

Further analysis of tomato juice was conducted after sterilization (heating, removal of the skin). The stability parameters of the juice such as gross viscosity (GV), serum density (SD), and precipitate weight ratio (PWR) were analyzed. The results for such analyses conducted with H9478 are given in **Table 4**. None of the parameters analyzed showed any significant changes in response to P fertilization.

Table 1. Effect of Hydrophos and Seniphos application on the yield of H9478 and H9997 tomatoes.

Cultivar	Treatment	Yield, Tons/ha
H9478	Control (Regular P)	170 ± 40
	Hydrophos (Regular)	198 ± 35
	Hydrophos (high conc.)	207 ± 21
	Seniphos (Regular)	190 ± 30
	Seniphos (high conc.)	227 ± 47
H9997	Control (Regular P)	150 ± 25
	Hydrophos (Regular)	165 ± 30
	Hydrophos (high conc.)	178 ± 28
	Seniphos (Regular)	148 ± 20
	Seniphos (high conc.)	192 ± 23

Note: ± figures are standard deviation (SD)

Table 2. Physico-chemical parameters of H9478 unprocessed juice.

Treatments	P, mg/100g	Brix, °	a/b	Acidity,%	NSS, %	Ash, %
NP	103.60±15.66	5.35±0.19	1.69±0.31	0.37±0.03	5.77±0.23	1.05±0.23ab
RP	108.43±5.86	5.40±0.38	1.62±0.40	0.37±0.02	5.94±0.51	0.95±0.18b
Hydro	101.92±10.90	5.40±0.36	1.81±0.13	0.38±0.02	5.96±0.50	1.11±0.15ab
Seni	110.32±9.88	5.23±0.39	1.81±0.13	0.39±0.02	5.73±0.38	1.02±0.16ab
LP	114.94±27.82	5.10±0.62	1.72±0.24	0.38±0.04	5.68±0.42	1.24±0.27a
HP	113.47±16.08	5.08±0.45	1.76±0.07	0.39±0.03	5.75±0.69	1.24±0.24a

Table 3. Physico-chemical parameters of H9997 unprocessed juice.

Treatments	P, mg/100g	°Brix	a/b	Acidity,%	NSS, %	Ash, %
NP	90.99±5.59b	5.00±0.56	2.03±0.06	0.39±0.03	4.88±0.55	0.86±0.25
RP	97.08±7.47ab	4.83±0.36	2.10±0.00	0.36±0.04	5.05±0.48	0.89±0.16
HYDRO	92.25±4.36b	4.83±0.60	2.00±0.03	0.36±0.04	4.94±0.47	0.79±0.05
SENI	92.88±3.73b	4.73±0.26	1.98±0.01	0.39±0.02	4.88±0.21	0.85±0.15
LP	98.13±6.11ab	5.08±0.71	1.99±0.11	0.37±0.02	5.18±0.52	0.96±0.19
HP	101.71±4.95a	4.95±0.06	2.10±0.08	0.38±0.02	4.82±0.41	0.95±0.09

Table 4. Physico-chemical parameters of processed juice from H9478.

Treatments	°Brix	Lycopene, mg/100g	a/b	GV, mPa.s	SD, g/mL @ 24ºC	PWR, %
NP	5.50±0.18	18.17±3.24	1.39±0.10	1150±87	1.0265±0.003ab	13.77±0.63
RP	5.63±0.48	17.98±1.53	1.40±0.04	1150±129	1.0288±0.003ab	14.40±0.71
Hydro	5.65±0.47	18.42±1.84	1.40±0.05	1200±163	1.0292±0.003a	14.18±1.30
SENI	5.35±0.44	16.70±2.42	1.36±0.10	1175±96	1.0265±0.003ab	14.11±1.41
LP	5.28±0.55	17.32±3.03	1.37±0.06	1075±189	1.0251±0.002b	13.47±0.39
HP	5.40±0.59	16.11±2.78	1.33±0.10	1175±171	1.0268±0.003ab	14.46±1.17

Similar studies were conducted with juice obtained from H9997 tomatoes. As in H9478, no major effect was observed as a result of P supplementation (**Table 5**). Further analyses were conducted with sauce preparation from H9478 and H9997 tomatoes. The sauce was concentrated to a Brix level of 9. The results are shown in **Tables 6 and 7**. The red color (a), lycopene levels, the red to yellow ratio (a/b), gross viscosity, serum density and precipitate weight ratio did not show any major changes in response to P supplementation. However, in H9997 tomato sauce preparation, an increase in gross viscosity was observed in response to Hydrophos and Seniphos application (**Table 7**).

Discussion

The results from the 2002 season did not show major differences in response to P fertilization in most of the parameters analyzed. This could be due to several factors, including ideal growing conditions, the presence of high soil P, etc. Even under conditions when P was not supplied (NP), the yield and other

Table 5. Physico-chemical parameters of processed juice from H9997.

Treatments	°Brix	Lycopene, mg/100g	a/b	GV, mPa.s	SD, g/mL @ 24ºC	Acidity, %
NP	5.13±0.47	17.39±1.51	1.53±0.09	1400±141	1.0412±0.003	0.39±0.03a
RP	4.90±0.42	16.80±3.00	1.51±0.12	1375±126	1.0351±0.003	0.36±0.04b
Hydro	5.00±0.62	17.90±1.12	1.54±0.07	1375±125	1.0324±0.002	0.36±0.04b
Seni	4.83±0.21	16.22±1.29	1.48±0.04	1400±469	1.0367±0.006	0.38±0.02ab
LP	4.50±1.89	17.53±3.10	1.51±0.11	1200±216	1.0382±0.006	0.37±0.02ab
HP	4.95±0.10	17.97±1.80	1.53±0.10	1250±100	1.0358±0.003	0.38±0.02ab

Table 6. Physico-chemical parameters of processed sauce with 9°Brix from H9478.

Treatments	a+	Lycopene, mg/100g	a/b	GV, mPa.s	SD, g/mL @ 24ºC	PWR, %
NP	32.24±3.63	23.26±5.87	1.35±0.06	6600±712	1.0479±0.005	21.69±2.08
RP	33.07±2.61	22.13±3.07	1.27±0.19	6400±1030	1.0478±0.005	22.31±2.63
Hydro	33.75±1.35	23.04±3.96	1.28±0.18	6500±739	1.0446±0.002	21.49±2.68
Seni	34.07±1.11	23.29±4.29	1.16±0.13	7475±900	1.0455±0.003	21.89±3.79
LP	34.12±1.99	24.12±3.24	1.28±0.12	7075±1300	1.0451±0.005	21.89±2.30
HP	32.51±1.07	24.05±3.70	1.23±0.16	7475±1866	1.0469±0.003	23.11±2.69

Table 7. Physico-chemical parameters of processed sauce with 9ºBrix from H9997.

Treatments	Acidity, %	Lycopene, mg/100g	pH	GV, mPa.s	SD, g/mL @ 24ºC	PWR, %
NP	0.62±0.08	29.50±4.49ab	4.19±0.04	9950±1399	1.0412±0.003	22.93±0.52
RP	0.57±0.02	29.87±5.58b	4.25±0.02	11500±3185	1.0351±0.003	22.25±0.81
Hydro	0.62±0.08	32.32±5.19ab	4.26±0.04	12150±1350	1.0324±0.002	23.73±0.86
Seni	0.65±0.03	30.37±2.97ab	4.24±0.07	12200±849	1.0367±0.006	23.49±0.73
LP	0.58±0.05	33.96±7.46a	4.24±0.04	11500±3169	1.0382±0.006	23.92±1.17
HP	0.59±0.03	29.52±5.55ab	4.27±0.04	10500±1740	1.0358±0.003	22.81±1.44

physicochemical parameters were similar to those supplied with P. The higher yield observed in NP for H9478 could be an experimental variation and is not statistically significant. By comparison to the two previous seasons, the yield was more than doubled during the 2002 season. The growing conditions were not ideal during the 2000 and 2001 season, and yield increases were observed in response to P fertilization. Thus, it appears that effect of P application becomes pronounced mostly under stressful conditions, where the availability of extra P could result in added stress protection. In support of this hypothesis, we have observed higher levels of antioxidant enzyme activities in tomatoes from P supplemented plots. During 2002 season, we also studied the effects of P supplementation on a high lycopene tomato variety H9997. Though the lycopene levels in the processed juice were similar between H9478 and H 9997, the lycopene levels appeared to be higher in the sauce preparation of H9997 tomatoes.

As observed in previous years, Hydrophos and Seniphos application appears to show positive results. In our earlier experiments, we have used a more diluted preparation of these formulations. Irrespective of the concentration differences, Hydrophos application tends to enhance the yield slightly. The effect is more pronounced with Seniphos, and we have observed these beneficial effects in the previous seasons as well. Improvements are noticeable in the quality of processed products as well.

Further analyses on vitamin C levels, antioxidant enzymes, flavor profiles, etc. are being conducted.