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STUDY OF THE OPTIMAL PRODUCTION PROCESS AND APPLICATION OF APPLE FRUIT [*MALUS DOMESTICA* (L.) BORKH] FERMENTATION

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Abstract

In orchard production, fruit abscission is common due to insect damage, disease, crop thinning and natural dropping. However, the utilization of these discarded plant resources has received little research attention. In this study, we used apple fruit from such plant resources, mainly young and mature dropped fruit, as materials and mixed them with a fermentation agent, brown sugar and water. The effects of the proportion of fermentation agent and the fermentation conditions (O_2, temperature, fermenting time and fruit crushing degree) were studied using an orthogonal experimental design. We discovered a novel fermented fertilizer, apple fruit fermentation nutrient solution (AFF), for which the optimal fermentation formula and conditions were comminuted young apples: fermentation agent: brown sugar: water weight ratio of 5:0.1:1:4 and 45 days of aerobic fermentation. Analysis of the fermentation solutions showed that the supernatant obtained using these optimized parameters had the highest mineral element content among the fermentation formulas and conditions studied. The results of a spraying experiment with 200-, 500- and 800-fold dilutions showed that AFF significantly promoted the net photosynthetic rate, leaf area and thickness, specific leaf weight, and chlorophyll and mineral element content in the leaves of young apple trees relative to the control treatment. The effects of 200-fold diluted AFF on the photosynthetic rate, the developmental quality and mineral element contents were greater than those of the 500- and 800-fold dilutions. The results of the spraying of adult trees with 200-fold diluted AFF compared to a water control demonstrated that AFF significantly enhanced the average weight of a single fruit, the shape index, hardness, content of soluble solids, titratable acid content, vitamin C content, and aroma compound content of the fruit of the adult trees. This evidence suggests that the AFF obtained using the optimal production process could effectively improve the vegetative growth and fruit quality of apple trees.

Key words: Apple fruit fermentation, Mineral elements, Photosynthesis, Leaf properties.

Introduction

In orchard production, flowers and fruit frequently drop and are thinned to regulate the tree loads, and shoots and stems are pruned to improve the canopy light conditions. The discarded materials, if not removed immediately, can lead to significant soil pollution, pest invasion, disease transmission and other environmental problems, hindering tree growth and development. To effectively utilize these waste plant resources, farmers often use the pruned stems and shoots to manufacture organic fertilizers each year before winter, while the thinned or naturally dropped fruits remain on the surface of the orchard soil, affecting normal soil management practices. An increasing number of studies have attempted to transform these materials into fertilizers via rapid fermentation (e.g., Han, 2006). Such efforts include the selection of appropriate primary and auxiliary materials, microbial fermentation agents and carbon resources as well as their proportions and the instrument settings, means of material processing and fermentation conditions. Such fermentation nutrient solutions, used as foliar fertilizers, feature high contents of mineral elements, sugars, amino acids, proteins and plant hormones as well as beneficial microbes, which improve the plant nutrient levels during leaf and fruit growth and development after rapid absorption by fruit trees.

Most studies concerning the application of plant-derived solutions have focused on the effects of extracts from specific plants, such as Chinese medicinal herbs, on the nutrient levels and the prevention of pests and diseases in horticultural crops. For example, spraying extracted rhubarb (*Rheum palmatum*) solution on the surface of leaves contributed substantially to preventing the development of powdery mildew in cucumber plants (*Cucumis sativus* L.) (e.g., Tang *et al*., 2003). The supernatant extracted from euphorbia chamaejasme (*Euphorbia fischeriana* Steud.) plants was used as a spray for apple trees, decreasing the incidence of fruit cankers (Li *et al*., 2009; Dai *et al.,* 2008). In recent years, various studies have identified several plant-derived nutrient solutions obtained through fermentation that can be applied to fruit plants under organic production conditions to improve tree growth and fruit quality (e.g., Geng *et al*., 2011). Li *et al*. (2009) developed a method to produce a nutrient solution from the flowers and fruit of the *Pyrus* cv. Xueqing pear plant and the flowers of silver chain (*Robinia pseudoacacia* Linn.) and demonstrated that the use of this solution as a foliar fertilizer improved shoot growth, leaf area, fruit weight and soluble solids in pear trees. Our previous studies on the manufacturing of nutrient solutions using aromatic plants, such as basil, mint, and purple perilla, indicated that spraying these nutrient solutions on adult pear trees not only increased the levels of mineral nutrients in the leaves but also inhibited the spread of scabs, ring spots and Valsa cankers on pear fruit. The nutrient solution contained many beneficial microbes due to fermentation, such as *Bacillus subtilis*, which could produce a bio-surfactant under appropriate conditions, thereby decreasing the presence of plant pathogens and increasing the bioavailability of nutrients for beneficial plant-associated microbes in agriculture (e.g., Sachdev *et al.,* 2013).

However, the optimal production process for nutrient solutions from the fermentation of orchard plant residues remains unknown. With the development of organic production processes for orchards, it is increasingly necessary for the development and application of such nutrient solutions instead of chemical fertilizer to promote tree development and prevent the spread of disease. In this study, we selected apple fruit as the main material, brown sugar as the carbon source and a fermentation agent as a catalyst. Based on the total mineral content and proportion, we determined the optimal fermentation formulas and conditions (O_2, temperature, fermenting times and fruit crushing degree) using an orthogonal experimental design. Next, the physicochemical properties and nutrient contents of the fruit-derived nutrient solutions were analyzed, and the effects of their application on apple tree growth and fruit quality were assessed. The ultimate objective was to provide a practical nutrient solution, pesticide and antibacterial agent and production methods appropriate for organic fruit production.

Materials and Methods

Plant materials: Two types of apple fruit (*Malus domestica* cv. Fuji), young and mature dropped fruit, were used as materials for the nutrient solution fermentation. The properties of the young fruit were as follows: average weight of a single fruit (AWSF) > 15 g, total soluble solids (TSS) > 8% and titratable acid (TA) < 3.5%. The properties of the defective mature fruit were as follows: AWSF > 184 g, TSS > 11% and TA < 13%. All fruit were collected from the China-Japan Friendly Sightseeing Orchard in the Changping District of Beijing.

Spray experiments were conducted on two types of plants. The first experiments were conducted on 100 two-year-old apple trees (*M. domestica* cv. Fuji)/ (*M. micromalus* Maik) of equal vigor, which were planted in plastic pots (33.5 cm-28.5 cm) at the Beijing University of Agriculture in the spring of 2006. Each pot contained 15 kg of culture medium containing a mass ratio of garden soil, sand, peat and manure of 3:3:3:1. The second experiment was conducted on 12-year-old adult plants (*M. domestica* cv. Fuji)/ (*M. micromalus* Maik) at the China-Japan Friendly Sightseeing Orchard. The apple trees were planted at a spacing of 3 m × 5 m and trained into open-center form. All tree management followed the standards of organic fruit production.

Formulas for the apple fruit-derived nutrient solutions and treatments used in the spraying experiments: This studied used 12 formulas varying by fermentation agent, fruit type, and aerobic/anaerobic conditions. Before fermentation, all fruits were washed and sterilized with boiling water and then cut into lumps or chopped using a disintegrator. These pieces were then mixed with specific proportions of brown sugar, water, and two types of fermentation agent, Lvzhou fermentation agents #3 and #4 (Jiamusi Lvzhou Company, Jiamusi, Heilongjiang, China), containing *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus licheniformis*, *Weissella* and *Coprinus* mushrooms as the predominant strains (Table 1). The mixtures were then stratified into fermentation containers (i.e., glass column pots). The apple fruit, brown sugar and fermentation agent were mixed evenly. Next, clean well water was added, and the containers were sealed with plastic film with or without holes. During fermentation, the room temperature was maintained at approximately 25ºC, and the solution was stirred every 5-7 d until the end of fermentation at 45-50 d. The fermentation progress and nutrient composition were determined weekly.

The nutrient solution with the highest fertilizer level was selected for use in the spraying experiments. This solution was prepared in accordance with Formula No. 12 (Table 1) and fermented at 25°C for 45 d. For the young trees, we sprayed nutrient solutions diluted by factors of 200 (T1), 500 (T2) and 800 (T3), and water was used as a control (CK). For the adult trees, T1 and CK were applied every 10 d from June 10th to August 10th; T2 and T3 were not used. The experiment was conducted in a randomized block design, with each treatment replicated ten times (young trees) or three times (adult trees).

Table 1. Nutrient solution formulations. LZ refers to the Lvzhou fermentation agent.

Formula	Fruit type	Fruit weight (kg)	Fermentation agent type (#)	Fermentation agent (kg)	Brown sugar (kg)	Water (L)	Ae or An	L/C
No. 1	MF	2.5	3	0.05	0	1	Ae	L
No. 2	MF	2.5	4	0.05	0	1	Ae	L
No. 3	MF	2.5	3+4	0.05	0	1	Ae	L
No. 4	MF	0.50	4	0.01	0.10	0.20	Ae	C
No. 5	MF	0.50	4	0.01	0.10	0.20	Ae	L
No. 6	MF	0.50	4	0.01	0.10	0.20	An	C
No. 7	MF	0.50	4	0.01	0.10	0.20	An	L
No. 8	MF	2.5	4	0.05	0	1	Ae	L
No. 9	YF	2.5	4	0.05	0.50	1	Ae	L
No. 10	YF	0.50	4	0.01	0.05	0.50	Ae	C
No. 11	YF	0.50	4	0.01	0.10	0.50	Ae	C
No. 12	YF	0.50	4	0.01	0.10	0.40	Ae	C

MF and YF refer to mature fruit and young fruit, respectively. Ae, aerobic condition; An, anaerobic condition; C, comminuted fruits; L, fruit lumps

Determination of nutrient compositions and solution properties: At the midpoint and end of fermentation, we selected 10 ml of three lots of nutrient solution from every formula for investigation. The pH of the nutrient solution was measured using a PHS-25 acidometer (Shanghai Precision & Scientific Instrument Co., Ltd., China). The total soluble solids content was measured with a WYT-4 sugar-measuring instrument (Shanghai Precision Instrument Co Ltd.). The total acidity and VC content were measured using acid–base and 2,6-dichlorophenolindophenol titration methods, respectively. The soluble sugar (SS) content was measured using the anthracene ketone colorimetric method (Han, 1996; Li, 2000). The nitrogen content was measured according to the Kjeldahl method (e.g., Bao, 2000). The phosphorus content was measured using the vanadium-molybdenum-yellow colorimetric method (e.g., Bao, 2000). The available K, Ca, Mg, Mn, Fe, Zn and Cu were measured using an atomic absorption spectrophotometer (Hitachi Z-5000, Japan) (e.g., Bao, 2000).

Determination of leaf properties and nutrient compositions: Five leaves were selected from the middle of the shoots to determine the leaf properties and nutrient composition. The leaf area was determined using a LI-3000A leaf area analyzer (Li-Cor, Inc., Lincoln, Nebraska, USA), and the specific leaf weight was determined by weighing the material (e.g., Zhao *et al*., 2011). The leaf thickness was measured using a Vernier caliper (e.g., Zhao *et al*., 2011). The nitrogen content was measured according to the Kjeldahl method. Vanadate-molybdate-yellow colorimetry was used to determine the phosphorus content (Hao *et al*., 2004). The available K content was measured using an Fp-6410 flame photometer (Suzhou Jiangdong Precision Instrument Co., Ltd., China) (e.g., Hao *et al*., 2004). The available Ca, Mg, Mn, Fe, Zn and Cu were measured using a Hitachi Z-5000 spectrophotometer (e.g., Hao *et al*., 2004).

Determination of the leaf photosynthetic characteristics: Three functional leaves of the same age were chosen from every three shoots in saplings and adult trees every 2 h from 07:00 to 17:00 on sunny days. The net photosynthetic rate (Pn), stomatal conductance (Cr), intercellular CO_2 concentration (Cs) and transpiration rate (Tr) of the leaves from different treatments were measured using a CIRAS-1 portable photosynthesis analyzer (UK PP-System, Inc.) (e.g., Wang, 1994). The water use efficiency (WUE) was calculated as Pn/Tr (e.g., Zhao *et al*., 2011).

Determination of fruit properties: From each plant, 10 fruits were randomly taken from the southern periphery of the tree crown to determine the fruit properties. The fruits were weighed, and the average weight was calculated. The fruit size (longitudinal and transverse diameters) was measured using a Vernier caliper, and the fruit shape index was calculated as the longitudinal diameter/transverse diameter. The fruit hardness was determined with a GY-1-type hardness tester. The soluble solids content was measured using a WYT-4 sugar-measuring instrument. The total acidity and VC were calculated using acid–base and 2,6-dichlorophenolindophenol titration (Zhao *et al*., 2002; Guan *et al*., 2001), respectively.

Determination of fruit volatile components: The fruit volatile components were measured using a HPGC6890/ MS5973 (Agilent Technologies Co. Ltd., USA) gas chromatography/mass spectrometry instrument with a manual SPME injector and a 100-m polydimethylsiloxane (PDMS) extraction head to measure the adsorption of volatile substances in the fruit. The following chromatographic conditions were used: an HP-5 (30 m×0.1 mm×0.33 m) flexible quartz fiber capillary column, helium as the carrier gas, an injector temperature of 220°C, an MS interface temperature of 280°C and splitless injection. The following temperature program was used: the initial temperature of 80°C was maintained for 1 min, increased by 10°C/min to 100°C, maintained for 1 min, increased by 4°C/min to 200°C, maintained for 4 min, increased by 10°C/min to 2500°C, and maintained for 2 min. Thus, the program lasted a total of 40 min. The MS conditions were as follows: an ion source temperature of 15°C, EI mode, an ion energy of 70 eV, full-scan mode and a scan range of 29-540 µl. Data were collected using HP ChemStation software. The initial identification of components was carried out using the NIST library, but this information was then combined with the retention time, mass spectrometry data, actual composition data and retention indices. In this way, the identities of most of the aroma compounds were verified (Jirovetz *et al*., 2003; Sara *et al*., 2002; Wang *et al*., 2005).

Statistical analysis: All data were analyzed using one-way ANOVA followed by Duncan's shortest significant ranges (SSR) test to compare the differences among the experimental sites at a significance level of $p < 0.05$. Microsoft Excel 2003 and DPS 7.05 were used for the calculations.

Results

Effect of substrate composition and fermentation conditions on the quality of the derived nutrient solution: Using a formula of 2.5 kg of mature defective fruit, 0.05 kg of fermentation agent, and 1.0 L of water, we selected Lvzhou fermentation agents #3, #4 and #3+#4 for the fermentation treatments (formula nos. 1-3, respectively). Under aerobic conditions and fruit lumps, the nutrient solution with formula no. 2 contained significantly higher contents of mineral elements ($p<0.05$; i.e., N, P, Fe, Mn, Cu and Zn) relative to the other nutrient solutions (Table 2).

We designed four groups of treatments with fruit lumps or comminuted fruit and aerobic/anaerobic conditions based on 0.5 kg of defective mature fruit, 0.01 kg of Lvzhou fermentation agent #4, 0.10 kg of brown sugar and 0.2 L of water (formula nos. 4-7). Of these four nutrient solutions, the contents of all mineral elements except for Ca and Mg, TSS, SS and TA were higher when aerobic conditions and comminuted fruit plant material were used (Table 3 and Fig. 1e).

Table 2. Effect of different fermentation agents on the mineral element content of apple-derived nutrient solution.

Formula	N (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Fe (mg/L)	Mn (mg/L)	Cu (mg/L)	Zn (mg/L)
No. 1	0.3b	462b	900a	1305a	542a	20.23b	6.79b	2.30b	4.79b
No. 2	0.5a	574a	900a	835.4b	438b	27.86a	9.24a	3.3a	6.35a
No. 3	0.2b	345c	850b	1357.8a	418c	6.97c	5.10c	2.0b	2.10c

Substrates were fermented for 60 days at 25ºC. The supernatant was removed and subjected to atomic absorption spectroscopy to determine the ion concentrations. With all other conditions being the same, formula nos. 1, 2 and 3 contained fermentation agents #3, #4 and #3+#4, respectively

Table 3. Effect of different fermentation conditions on the mineral element content of young-fruit-derived nutrient solution.

Formula	N (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Fe (mg/L)	Mn (mg/L)	Cu (mg/L)	Zn (mg/L)
No. 4	0.47b	669.2a	2433a	3850c	2650b	219.8	8.33a	0.8c	5.3a
No. 5	0.42b	631.9a	1766b	4450a	2883a	159.5b	7.3b	0.7c	3.8b
No. 6	0.49b	584.3b	1450c	3350d	2383d	128c	5.43c	1.6a	5.2a
No. 7	0.58a	480.7d	1383c	4050b	2550c	119.2c	4.3d	1.1b	3.2b

Formula nos. 4, 5, 6, and 7 corresponded to conditions Ae/C, Ae/L, An/C, and An/L, respectively, with all other conditions being the same. Ae, aerobic condition; An, anaerobic condition; C, comminuted fruits; L, fruit lumps

Table 4. Effect of different fruit types on the mineral element content of apple-derived nutrient solution.

Formula	N (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	M (mg/L)	Fe (mg/L)	Mn (mg/L)	Cu (mg/L)	Zn (mg/L)
No. 8	0.2b	513b	1750a	1237.3b	634.6b	62.55a	1.39a	1.25b	0.60b
No. 9	0.5a	634a	1650b	3113.6a	1001.4a	43.26b	1.52a	6.31a	2.19a

Defective mature fruit were added without brown sugar in formula no. 4, while young apple fruit were added with 0.5 kg of brown sugar in formula no. 5. All other conditions were the same

Table 5. Nutrition content of 100 g of brown sugar.

Component	Content	Component	Content
Protein (g)	0.7	Carbohydrates (g)	96.6
Water (g)	1.9	Thiamine (μg)	0.01
Ash (g)	0.8	Vitamin PP (mg)	0.3
K (mg)	240	Na (mg)	18.3
Ca (mg)	157	Mg (mg)	54
Fe (mg)	2.2	Mn (mg)	0.27
Cu (mg)	0.15	Zn (mg)	0.35
P (mg)	11	Se (g)	4.2

It could be deduced from Table 4 that the mineral element contents of formula no. 9 were significantly higher than those of the solutions. To determine whether the brown sugar had an effect on the results, the composition of brown sugar was analyzed, revealing that the composition included a large number of minerals (Table 5). Because brown sugar was added to formula no. 9, this formula outperformed the formula with defective mature fruit. Thus, we found that formula nos. 8 and 9 were the most suitable.

A group of experiments was designed for the aerobic conditions with comminuted fruit. The basic formula used in these experiments was 0.5 kg of young fruit, 0.01 kg of Lvzhou fermentation agent #4, 0.5 L of water and 0.05 or 0.10 kg of brown sugar (formula nos. 10 and 11). The nutrient solution derived using less brown sugar had lower nutrient levels (e.g., TSS, SS, TA and mineral elements) and a higher pH (Table 6 and Fig. 1a). As the amount of brown sugar added to the AFF increased, the contents of the major elements increased, while the contents of the trace elements remained the same or decreased (Table 6 and Fig. 1c).

The two treatments using 0.4 and 0.5 L of water (formula nos. 11 and 12, respectively) were used to determine the optimal added water content using the basic formula of 0.5 kg of comminuted young fruit, 0.01 kg of fermentation agent and 0.1 kg of brown sugar under aerobic conditions. After fermentation, the 0.4-L treatment yielded higher TSS, SS and TA contents (Fig. 1d); significantly higher N, P, K, Ca, Mg and Fe contents ($p<0.05$); and significantly lower Mn, Cu and Zn contents ($p<0.05$; Table 7).

We used a basic formula of 2.5 kg of comminuted young fruit, 0.05 kg of Lvzhou fermentation agent #4, 0.5 kg of brown sugar, 1 L of water, and aerobic conditions with fermentation times of 15, 30, 45 and 60 d (formula no. 9). The TSS, SS, and TA ceased to change after 30 d of fermentation (Fig. 1b), and the pH was stable after 15 d of fermentation. The mineral element contents of the supernatant (i.e., K, Ca and Mg) increased for 45 d and then decreased (Table 8). The N, P, Fe, Mn and Zn contents increased rapidly in the first 45 d and then more gradually. The Cu content remained stable for 30 d.

To investigate the effects of fermentation type and conditions, defective mature apple fruits were chosen as the plant material. Our findings indicated that the formula with Lvzhou fermentation agent #4 under aerobic conditions was better than the other formulas when the fruits were comminuted during fermentation. Thus, the optimal formula was a 5:0.1:1:4 ratio of comminuted young fruit, fermentation agent, brown sugar and water, and the nutrient solution was aerobically fermented at 25°C for 45 d.

Table 6. Effect of the addition of different amounts of sugar on the mineral element content of young-fruit-derived nutrient solution.

Formula	N (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	M (mg/L)	F (mg/L)	Mn (mg/L)	Cu (mg/L)	Zn (mg/L)
No. 10	0.4b	654b	846b	2583b	1633b	119.7a	4.77a	0.8a	4.7a
No. 11	0.6a	723a	1150a	3266a	2066a	93.5b	4.87a	0.8a	4.7a

With all other conditions being the same, 0.05 and 0.10 kg of brown sugar were added to formula nos. 10 and 11, respectively

Table 7. Effect of the addition of different amounts of water on the mineral element content of young-fruit-derived nutrient solution.

Formula	N (%)	P (mg/L)	K (mg/L)	C (mg/L)	Mg (mg/L)	Fe (mg/L)	Mn (mg/L)	Cu (mg/L)	Zn (mg/L)
No. 11	0.6a	723a	1150a	3266a	2066a	93.5b	4.87a	0.8a	4.7a
No. 12	0.6a	713b	853b	2583b	1633b	94.93b	4.87a	0.8a	4.3a

With all other conditions being the same, 0.5 and 0.4 kg of water were added to formula nos. 11 and 12, respectively

Table 8. Effect of different fermentation times on the mineral element content of young-fruit-derived nutrient solution.

Fermentation duration	N (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Fe (mg/L)	Mn (mg/L)	Cu (mg/L)	Zn (mg/L)
15 days	0.60c	501.2b	1612a	4100a	2337b	70.4b	5.3c	1.8b	1.8b
30 days	0.65b	547.5b	1619a	4063a	2367b	82.5a	5.6b	2.2a	3.0a
45 days	0.66b	583.6a	1625a	4150a	2412a	84.38a	5.7b	2.3a	2.8a
60 days	0.67a	605.7a	1600a	4088a	2300b	85.38a	5.8a	2.3a	3.0a

Using formula no. 9, substrates were fermented for 15, 30, 45, and 60 days at 25ºC. The ion concentrations of the supernatant were determined using atomic absorption spectroscopy

Table 9. Leaf growth of apple trees sprayed with different concentrations of fruit-derived nutrient solutions.

Treatment	LA (cm^2)	LT (cm)	SLW (g/cm)	CHLa	CHLb	CHLa+b	Pn	CS	Ci	Tr
CK	26.01c	0.03a	0.013a	1.46c	0.74c	2.20c	10.55b	155.1a	86.3a	6.33a
D200	30.44a	0.04a	0.016a	1.82a	1.00a	2.82a	12.43a	124.5b	61.8c	5.66c
D500	28.11b	0.03a	0.016a	1.78a	1.02a	2.80a	10.73b	138.7b	76.3b	5.98b
D800	26.83c	0.02a	0.017a	1.67b	0.87b	2.54b	12.29a	152.4a	84.5a	6.26a

D200, D500 and D800 refer to nutrient solutions diluted by factors of 200, 500 and 800, respectively; LA, leaf area; LT, leaf thickness; SLW, specific leaf weight; CHL, chlorophyll content ($mg·L^{-1}$); Pn, net photosynthetic rate (μmol $CO_2/m^2·s$); Cs, stomatal conductance ($mmol·m^{-2}·s^{-1}$); Ci, intercellular CO_2 concentration (ppm); Tr, transpiration rate ($mmol.m^{-2}s^{-1}$)

Table 10. Mineral element content of leaves sprayed with different concentrations of fruit-derived nutrient solutions.

Treatment	N (%)	P (mg/l)	K (mg/l)	Ca (mg/l)	Mg (mg/l)	Fe (mg/l)	Mn (mg/l)	Cu (mg/l)	Zn (mg/l)
CK	2.19c	347c	1068ab	1273c	380.2c	267.3b	38.5b	9.3a	28.1a
D200	2.62a	603a	1120a	1250c	528.3a	459.7a	71.7a	13.7a	26.3a
D500	2.40a	456b	1087ab	1560a	406.6b	253.3b	44.3b	9.3a	22.7a
D800	2.38b	442b	1053b	1333b	360.5c	235.3b	46.7b	10.2a	31.3a

Effect of nutrient solution on tree vegetative growth and fruit quality: Based on the results described above, we produced a fruit-derived nutrient solution from 50 kg of comminuted young fruit, 1 kg of Lvzhou fermentation agent #4, 10 kg of brown sugar and 40 L of water. Fermentation occurred under aerobic conditions at 25-28°C for 45 d. To investigate the effect of the resulting nutrient solution on the leaf properties and fruit quality, we sprayed it onto young apple trees at 200-, 500- and 800-fold dilutions (T1-T3, respectively), using water as the CK.

Compared with CK, the mineral element contents (i.e., N, P, Fe, Mn, Cu, K and Mg) in the leaves of young trees treated with the nutrient solution were significantly higher ($p<0.05$; Table 10). The leaf area, specific leaf weight and leaf thickness were also higher (Table 9). The chlorophyll content and Pn, Cs and Ci levels were higher in leaves treated with the nutrient solution. However, the transpiration rate of the leaves treated with the nutrient solution was lower than that of the control leaves. Thus, the water usage efficiency of the treated leaves was higher. Among the three treatments, the application of a 200-fold dilution provides the greatest improvement of leaf properties, nutrient contents and photosynthesis (Table 9).

We treated mature apple trees with the 200-fold-diluted nutrient solution and found a significantly higher ($p<0.05$) average single fruit weight, soluble solids content and VC content compared with the CK. In addition, the contents of many of the aroma compounds were higher in the treated fruits. However, the content of α-farnesene in fruit treated with the nutrient solution was lower than that in fruit treated with the CK ($p<0.05$; Tables 11 and 12).

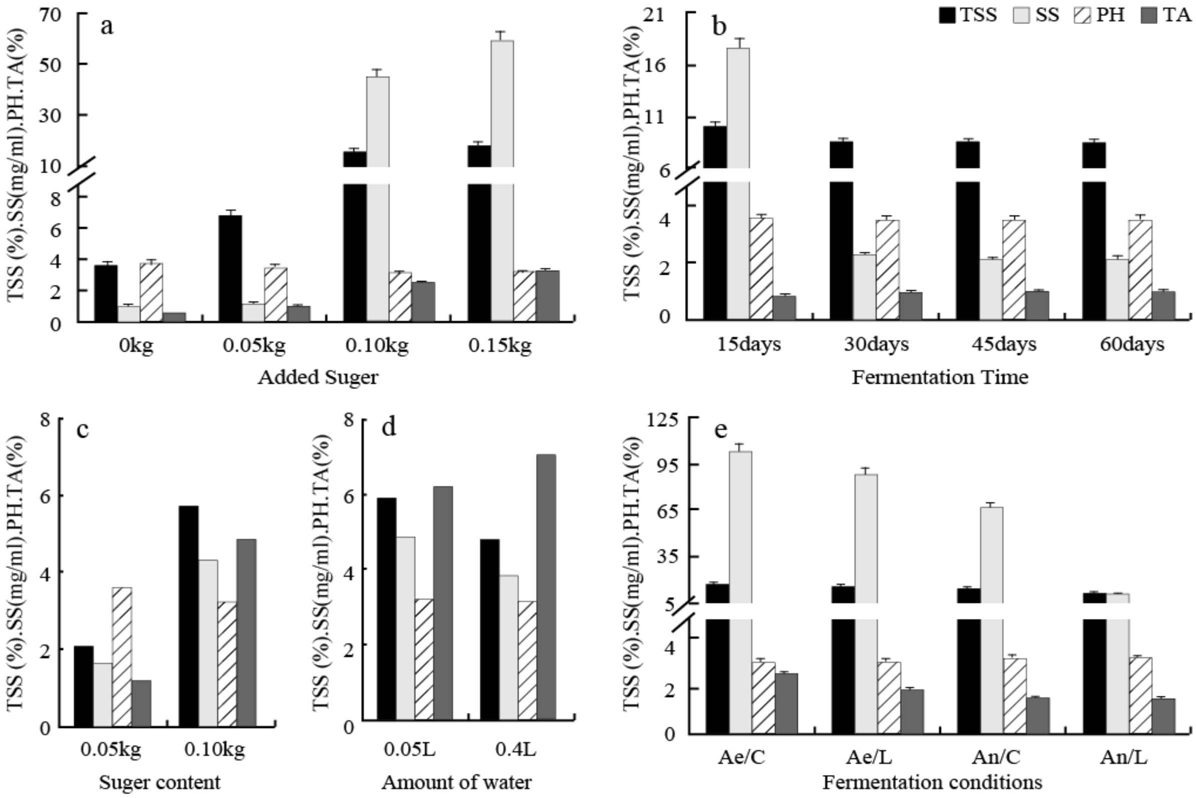

Fig. 1. Effect of different conditions on the physiochemical index of young apple-derived nutrient solution.
a. Effect of added sugar content on the physiochemical index of mature-apple-derived nutrient solution.
b. Effect of fermentation time on the physiochemical index of nutrient solutions.
c. Effect of added sugar content on the physiochemical index of young-apple-derived nutrient solution.
d. Effect of added water content on the physiochemical index of young-apple-derived nutrient solution.
e. Effect of different conditions on the physiochemical index of young-apple-derived nutrient solution.
TSS, total soluble solids (%); TA, titratable acid (%); SS, soluble sugar (mg/ml).

Table 11. Effect of different concentrations of fruit-derived nutrient solutions on apple fruit quality.

Treatment	WSF	VD	TD	FSI	FH	TSS	TA	Vc
D200	282.58a	74.13a	86.04a	0.86a	7.61b	14.5a	0.37a	2.38a
CK	236.68b	66.7b	82.21b	0.81a	6.42b	12.8b	0.36a	1.45b

AWSF, the average weight of a single fruit; VD, vertical diameter; TD, transverse diameter; FSI, fruit shape index; FH, fruit hardness; TSS, soluble solids; TA, titratable acid; Vc, vitamin C content

Table 12. Effect of different concentrations of fruit-derived nutrient solutions on the aroma compound content of apple fruit.

Treatment	AC-1	AC-2	AC-3	AC-4	AC-5	AC-6	AC-7	AC-8	AC-9
D200	7.12a	2.19a	13.30a	1.82a	2.37b	10.72a	3.20a	4.17a	33.88a
CK	6.13b	1.05b	3.73b	0.83b	28.52a	6.79b	1.55b	1.69b	28.08b

AC, aroma compounds: AC-1, butanoic acid, 2-methyl-, hexyl ester; AC-2, hexanoic acid, hexyl ester; AC-3, 7-methoxy-2,2,4,8-tetramethyltricyclo[5.3.1.0(4,11)]undecane; AC-4, butanedioic acid, bis(2-methylpropyl) ester; AC-5, α-farnesene; AC-6, 1-butanone, 1-[2,4,6-trihydroxyphenyl]; AC-7, butanedioic acid, methyl-, bis(1-methylpropyl) ester; AC-8, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester; AC-9, dibutyl phthalate

Discussion

In the fermentation of fruit-derived nutrient solution, internal and external factors can affect both the process and the quality of the final product. Internal factors might include the fruit types, the fermentation agent, and the proportions of the carbon source and water. External factors might include the degree to which the fruit were ground, whether the conditions were aerobic or anaerobic, and the temperature and duration of fermentation. In the present study, we found that young fruit were more easily fermented than mature defective fruit; the young-fruit-derived solutions contained more nutrients and thus improved the leaf photosynthesis levels and fruit quality of young trees. Lvzhou fermentation agent #4 accelerated fermentation, completely decomposed organic matter and increased the contents of the major mineral elements. Brown sugar was crude extracted from plants and contained a large number of minerals and other nutrients, which not only increase the sugar content but also improve the mineral element content in the supernatant of the fermentation broth. The mineral element content was highest when the mass ratio of young fruit to sugar was 5:1 and the mass ratio of mature defective fruit was 10:1, and the pH decreased slightly with increasing amount of sugar added during fermentation. The K, Ca and Mg contents did not increase over the first 45 d of fermentation, and their concentrations were related to the quantity of sugar used in the nutrient solution. Notably, the quantity of added sugar should differ by the amount of plant material obtained from different species, tissues or developmental stages in the fermentation system. For example, Dai *et al*., reported that the proportion of plant material to sugar was 1:1 in the fermentation of pear-flower-derived nutrient solution, which improved shoot growth, leaf area, fruit weight and soluble solids content (e.g., Dai *et al*., 2008). In a previous study, we found that the proportions clearly affected the fermentation of aromatic plant-derived nutrient solution; when the proportion of plant material to sugar was 40:3, large amounts of mineral elements, organic nutrients and hormones accumulated in the nutrient solutions, which might improve the growth of trees, the mineral nutrient contents of the leaves, and fruit quality and prevent leaf and fruit diseases in pear trees (e.g., Geng *et al*., 2011). Furthermore, the appropriate choice of comminuted fruit maturity, fermentation conditions and temperature could promote fermentation. The results of the present study suggest that the optimal conditions for the young-fruit-derived nutrient solution were a solution of comminuted young apple fruit mixed with fermentation agent, brown sugar and water (in a mass ratio of 5:0.1:1:4) and aerobic fermentation for 45 d at 25°C.

In the present study, the fermentation of fruit-derived nutrient solution was adapted from the traditional method of using persimmon vinegar and wine without adding any chemicals. The nutrient solution was shown to be safe and effective. The spraying of fruit-derived nutrient solution on young apple trees increased the mineral element contents (i.e., N, P, K, Ca, Mg, Fe, Mn and Cu), improving the photosynthesis rate, leaf area, leaf thickness, specific leaf weight and chlorophyll content. The net photosynthesis rate and water usage efficiency of the leaves were also improved. Spraying the nutrient solution on mature apple trees improved the fruit quality in terms of the average weight of a single fruit, fruit shape index, fruit hardness, soluble solids, titratable acid and VC content. The nutrient solution also increased the level of aroma components related to the fruit flavor, such as 1-butanone, 1-[2,4,6-trihydroxyphenyl], methyl-, bis(1-methylpropyl) ester, butanedioic acid, 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester and dibutylphthalate. The fruit quality improvement resulting from spraying the nutrient solution was mainly due to three factors. First, the nutrient solution contained abundant nutrients, which might be well suited for direct absorbance and utilization by fruit trees, improving the nutrient absorption and assimilation in the leaves. As a result, the leaf growth, net photosynthetic rate and water use efficiency of leaves may increase, increasing the amount of the substance accumulated in the plants. Second, we deduced that the nutrient solution might contain high levels of antioxidants (e.g., VC), physiologically active substances (e.g., polyamines) and plant hormones (e.g., IAA, GA and ABA). These active substances can regulate development and enhance the adaptability of trees in stressed environments. A study of an aromatic plant-derived nutrient solution showed that these substances were effective in improving the growth and development of trees due to their high levels and appropriate proportions of phytohormones (e.g., Geng *et al*., 2011). Finally, our laboratory found that the fruit-derived nutrient solution was weakly acidic and contains many antimicrobial substances, with clear exterior antimicrobial effects on *Venturia pirina* Aderh., *Physalospora pinicola* Nose. and *Valisa mali* in young pear trees. It was also possible that treatment with the nutrient solution might induce disease resistance in plants or tissues by improving the antioxidant ability of cells infected with pathogenic bacteria (e.g., Jiang *et al*., 2010). We previously mentioned that spraying nutrient solution could increase the content of aroma components and decrease the content of α-farnesene, which is related to a low incidence of apple scald on fruit (e.g., Zhang, 1991). These effects can improve fruit quality and storage properties and extending the shelf life of apples.

Conclusion

Nutrient solutions offer the ability to promote growth and development in fruit plants, but their production process is a complex technical system. We believe that our results will advance orchard production by creating nutrient solutions from young or mature defective fruit to safely improve fruit quality. The mechanisms of action of fruit-derived nutrient solutions on fruit trees will be further studied in the future.

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