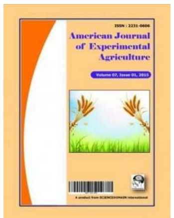

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Foliar Spray of Rice Rinsed Water to Tropical High Elevation Grown Strawberry (*Fragaria ananassa* Duch.) Increased Sugar Content of Ripe Fruits

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Authors' contributions

This work was carried out in collaboration between both authors. Author IGAMSA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author IGMON reviewed the experimental design and all drafts of the manuscript. Author IGMON performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

High air temperatures coupled with low light intensity, especially during the last two weeks of fruit ripening, may cause a low sugar content of ripe fruits of high altitude grown strawberry (*Fragaria ananassa* Duch.) in the tropics. An experiment in a plastic house was conducted to examine the effects of two week leaf spray, of rice rinsed water (RRW) containing carbohydrates on sugar content of ripe fruits. Preliminary investigation to measure the size of stomata opening in leaves of strawberry, the size of starch cells and the sugar and starch content in RRW of five varieties of rice, were also conducted. The main experiment in a plastic house, consisted of spraying to fruiting strawberry, five concentrations of RRW viz. 0, 5, 10, 15 and 20 ml l^{-1}, once a day at 9.00 – 10.00 p.m. Treatments were replicated six times in a randomized complete block design. Rice rinsed water (1.0 g rice: 2.0 ml water) from C_4 variety of rice contained 778.9 and 1494.7 μg ml^{-1} sugars and starch, respectively. Stomata opening was 20.25 x 9.36 μm, was larger than starch cells size of

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6.0 x 8.1 μm. Average minimum and maximum temperatures during the experiment were 15.0 and 25.8℃, respectively. No foliar disorder was observed resulting from the spray. Soluble solid content (SSC) of ripe fruits in plants receiving 10 ml l^{-1} leaf spray was 5.6%, increased (P = 0.05) 26.08% compared to control 4.6%, while sugars content increased 56.08% from 21135 to 32978 μg g^{-1} fresh weight. Further increased in concentration of RRW had no effect on SSC and sugar content of fruits. Relationships between concentrations of RRW with SSC and sugar content (μg g^{-1} fresh fruits) were quadratic.

Keywords: Rice rinsed water; foliar spray; sugars; strawberry fruits.

1. INTRODUCTION

Quality and price of strawberry fruits are largely determined by sugar content of the fruits [1-3], while sugar content of fruits was determined by genetic and supply of sucrose from leaves to fruits, especially during two weeks period of fruit maturity [4].

In the tropics of Bali, strawberry was planted in higher altitude (1000 m above sea level) areas, which have cooler temperatures suitable for the growth of the plants. However, air temperatures in the tropics were still higher than in the sub-tropics especially during fruits maturity in early spring in the sub-tropics. Higher temperatures increase the rates of dark respiration which requires sucrose resulted in a lower supply of sugars to fruits. In addition, clouds often cover the sky in higher elevation in the tropics leading to lower intensity of light intercepted by leaves [5]. Chandler et al. [6], Del Pozo-Insfran et al. [7] and Correia et al. [8] found that at certain season or if the fruits were harvested at the late season, the fruits contained less sugars and tasted sour which were attributed to more cloudy sky or warmer temperatures or larger crop loads. Wang and Camp [9] found that temperature optimum for highest fruit sugar content was 25/12℃ day/night temperatures, above that temperatures sugar content would decrease. MacKenzie et al. [10] also found that at constant temperatures of 15℃, strawberry fruits contained 6.5% soluble solid content (which was ca. 70% sugar), while at temperature 25℃ it was 5.2%, attributable to higher requirement for carbohydrate for dark respiration. Acuna-Maldonado and Pritts [11] found an increase of dry weight of leaves, shoots, whole plants and fruit weight of 28%, 17%, 21% and 17%, respectively as a result of increasing CO_2 concentration around the plants during the previous season. However, in their experiment the SSC and soluble sugar content of ripe fruits were not measured.

Rice rinsed water obtained during cooking rice in most of Indonesian households which is generally wasted, may contain soluble sugars, starch, and minute amounts of other substances. Sugars are dissolved in water and are readily transported in the plants, but sugars decrease water potential in the cells thereby disrupting normal physiological functioning of the cells which is not the case with starch. Girdling experiment showed that the leaves above girdled stem turned yellow which were caused by disruption of chloroplast in the leaves resulting from accumulation of sucrose [12].

The size of rice starch cells was smaller compared to cell size of other starch cells (corn, potatoes, sweet potatoes) and has been found to be smaller than the size of stomata opening of leaves of grasses, mangoes and *Gliricidia* spp. (Nurjaya unpublished data), thus can passively enter the leaves and transported in the plants through apoplast. In the leaves the starch will be stored temporally in the chloroplasts or vacuoles. During the night with the action of enzymes, starch will be transformed back to sugars and transported to other parts of the plants including fruits, thus increasing their sweetness.

Sucrose is the main soluble carbohydrates transported from leaves to other parts of the plant. During fruiting, competition for sucrose among different parts of plants exist [13], sucrose is used for respiration, growth of different organs or store as starch in organs (roots, stems). If the carbohydrates reserves in storing organs are low, the growth of new shoots in the next season will be impaired, the formation of fruits for the next harvest reduced and harvest declined. Reducing leaf areas during fruit ripening by removing [14] or shading [15] leaves will reduce fruit sugar content. Ordinary sugars (cane sugar) and rice flour can be used instead RRW, but their use will compete with other purposes. In addition, the use of cane sugar will disrupt leaf cell water potential.

Strawberry, as also grapes, apple and orange are C_3 plants (with ribulose diphosphate carboxylase enzyme fixing CO_2 in the leaves) widely planted in suitable areas of Indonesia, however, their fruit sugar content is generally low compared to similar imported fruits leading to their use as fruit juice with added sugars rather than as table fruits. The experiments reported here are intended to address the problem.

2. MATERIALS AND METHODS

2.1 Measurements of Stomata Openings in Strawberry Leaves and the Size of Rice Starch Cells

The size of ten stomata openings in strawberry leaves and the size of ten rice starch cells were measured in Common Research Laboratory, Faculty of Mathematic and Natural Sciences Udayana University, Jimbaran, Bali, Indonesia. Aceton was polished on the upper and lower surface of strawberry leaves at 10.00 a.m. After drying, the surface leaves with aceton were collected and microscope objects were made and the size of stomata openings were measured with the aid of a micrometer. Microscope objects containing rice starch cells were also made and the size of starch cells were measured as for stomata openings.

2.2 Analysis of Sugars and Rice Starch Content in Rice Rinsed Water of Some Varieties of Rice

2.2.1 Location and time of experiment

Analysis of sugars (glucose, fructose, sucrose and other sugars dissolve in 90% ethanol) and starch content in RRW of five varieties of rice sold in local markets in Denpasar were conducted in Analytical Laboratory Udayana University, Jimbaran, Bali.

2.2.2 Material and methods

Five varieties of rice commonly cooked in average household i.e. (A) Bengawan rice, (B) Super C4 rice, (C) Cangkul brand rice, (D) Sudaji Bali rice, and (E) Sehat Bali rice, were analysed.

100 g of rice was mixed with 200 ml of reverse osmosis (RO) water, thoroughly mixed for five minutes, and sieved with kitchen sieve, and the volume of rice rinsed water was made to 250 ml. 2 ml of RRW were mixed with 8 ml 80% ethanol in 15 ml centrifuge tubes boiled for 15 minutes in a water bath. Centrifuge at 2000 rpm for 10 minutes, supernatant was separated, the volumes were made to 25 ml. Used 1 ml for phenol test [16] with sucrose as standard. The pellet were dried and used for analysis of starch using acid hydrolysis methods and glucose as standard [17]. Two measurements were made for each variety of rice.

2.3 Experiment: Spraying of Rice Rinsed Water to Leaves of High Elevation Grown Strawberry Plants in the Tropics of Bali

2.3.1 Time and location of the experiment

The experiment was conducted in the village of Pekarangan, Pancasari, Bali (1000 m above sea level, 80 km north of Denpasar), in a plastic house from August 5th to August 24th, 2014. Minimum and maximum temperatures during the experiment were recorded with minimum and maximum thermometer placed ca. 0.60 m above the plants. The strawberry plants were grown in planting media in plastic rolls placed on three stages of concrete benches 21.0 m long, and 0.20 m wide. The benches were at east-west direction. Each bench consisted of three levels benches, two benches at the bottom row (left and right), two benches (left and right) in the middle row, and one bench at the upper row.

Planting media wrapped in black plastic rolls 0.80 cm length and 25.0 cm diameter with holes for planting, were placed on each bench. In each bench, there were 26 plastic rolls with small plastic pipe inserted to automatically watering the media with nutrients from 1000 l reservoir, by mean of a machine pump. Planting distance on the bench was 0.25 x 0.45 cm.

Planting media consisted of half burned rice hulls black in color. Watering was conducted once a day (before 9.00 a.m.) with 1600 ml nutrient solution A which is a organic fertilizer solution and 1600 ml solution B (Soil Plus) diluted in 700 ml of water in a big tank. Solution A contained C-organic 21.42%; N-total 0.84%; P_2O_5 0.96%; K_2O 1.16%; Cu 84.7 ppm; Zn 62.9 ppm, Mn 58.4 ppm; Fe 106.1 ppm; B 62.7 ppm, with pH 5.0.Solution A also contained *Azospirillum* sp., *Pseudomonas* sp., *Rhizobium* sp., *Bacillus* sp., and *Azotobacter*. Solution B is a complete organic fertilizer solution (Soil Plus) with composition of C-organic 4.5%; N-total 0.5%; P_2O_5 1.5%;, Cu 0.2 ppm; Zn 9 ppm; Mn 22 ppm;

Fe 101 ppm; MgO 0.03 ppm, Co 0.2 ppm; with pH 7.5.

Strawberry variety planted was Rosalinda variety from California, USA, an everbearing variety, one and half year old, started fruiting at three months old with ripe fruits harvested every three days. Development of flower to fruit maturity was ca. 30 days, and from fruit maturity to ripening for commercial picking was ca. two weeks.

2.3.2 Treatments and design of experiment

Treatments consisted of spraying the leaves with RRW in the concentration of 0, 5, 10, 15, 20 ml l^{-1}, with six replications in a randomized complete block design. Rice was rinsed with RO water in 1:2 (rice/water), mixed for five minutes, and the water was separated from the rice by mean of a kitchen sieve.

Blocks were selected as homogenous as possible in term of the size of the plants and number of flowers and fruits. The size of plots in blocks were 0.80 x 0.90 cm. Spraying was conducted once a day between 9.00 – 10.00 am with 150 ml rice rinsed solution until all leaves from the bottom and upper surface were wet, and water started to drip. After spraying period of two weeks, the plants were undisturbed for six days before all fruits were harvested, and stored below 5℃. Fruits were separated into (a) young green fruits which were fruits from petal fall to full size, (b) turning pink which were full size fruits starting to turn pink to fruits with two third pink colors, and (c) ripe fruits viz. fruits with full pink color suitable for commercial picking.

From each plot, the biggest ripe fruit was selected, the fruit was washed with RO water and bolted dry with tissue paper, cut into two parts, one part was used to measure SSC (Brix) using hand held refractometer (Artago, Japan). Fruit was crushed with a mortar and pestle, and the solution so obtained was read in a refractometer. Before and after reading the refractometer was standardized using RO water. The other half of the fruit was weighed circa 1.0 g extracted with 10.0 ml boiling 95% ethanol for 15 minutes to measure ethanol soluble sugars and starch. After extraction the solution was kept in plastic container in a refrigerator, and analyzed as soon as possible. Part of the ripe fruit remaining was weighed and ovendried for dry weight determination. Ethanol soluble sugars were analyzed following DuBois [16] with sucrose as standard and starch content was analyzed following acid hydrolysis [17] followed by phenol test with glucose as standard. Data were analyzed with Costat Statistical Software [18] and mean separations were conducted with Duncan's Multiple Range Test.

3. RESULTS

3.1 Ethanol Soluble Sugars and Starch Content of Rice Rinsed Water

Ethanol soluble sugars and starch content of RRW of five variety of rice commonly sold in Denpasar were presented in Table 1.

Sugar concentrations varied from 778.9 μg ml^{-1} in Super C4 rice variety (*Oryza sativa* cv. Super C4) to1939.6 μg ml^{-1} Sehat Bali rice variety, while starch content varied from 1494.7 μg ml^{-1} in Super C4 variety to 2243.9 μg ml^{-1} in Bengawan variety of rice.

Table 1. Ethanol soluble sugars and starch content (μg ml^{-1}) of rice rinsed water of five variety of rice commonly sold in Denpasar (means of two measurements)

No.	Variety of rice	Content (μg ml^{-1})	
		Sugar	Starch
1.	Bengawan	1476.4	2243.9
2.	Super C4	778.9	1494.7
3.	Cangkul brand	1008.3	1703.7
4.	Sudaji Bali rice	804.7	1888.4
5.	Sehat Bali rice	1939.6	1738.5

3.2 The Size of Stomata Opening and Size of Rice Starch Cells

Stomata were only found on the bottom surface of strawberry leaves with mean stomata opening of 20.25 μm in length and 9.36 μm wide. The mean size of rice starch cells was 6.6-8.1 μm in length and 6.0 - 7.3 μm wide.

3.3 Main Experiment: Strawberry Leaves Spraying with Rice Rinsed Water

3.3.1 Minimum and maximum temperatures during experimentation

Daily minimum and maximum temperatures during experimentation were presented in Table 2.

Table 2. Daily minimum and maximum temperatures (℃) during experimentation

Days	Temperature (°C)	
	Minimum	Maximum
1	16.5	31.0
2	16.0	27.0
3	16.5	27.0
4	16.5	24.5
5	16.5	26.0
6	14.0	27.0
7	12.0	24.0
8	14.0	26.0
9	15.0	24.5
10	16.5	24.5
11	15.0	21.0
12	14.5	26.0
13	12.5	26.5
14	15.0	26.0
Mean	**15.0**	**25.8**

Daily minimum air temperature in the plastic house varied from 12.5–16.5℃, while daily maximum temperature varied from 21.0–31.0℃. Mean daily air temperature varied from 15.0–25.8℃.

3.3.2 Fruit soluble solid and ethanol soluble sugar content

During the experiment there was no leaf disorder observed resulting from leaf spraying. Spraying with 10 ml l^{-1} RRW increased SSC (Brix) 5.8%, increased (*P*=0,05) 26.08% compared to control (spraying with RO water only) 4.6% (Table 3), while ethanol soluble sugars 32978 μg ml^{-1}increased (*P*= 0,05) 56.08% compared to control 21135 μg ml^{-1} (Table 3). Further increase in concentration of RRW had no effects on SSC and ethanol soluble sugars. No difference was observed in ethanol soluble sugars content on the basis of ripe fruit dry weight (Table 3). Relationships between concentration of RRW and SSC (Brix) and ethanol soluble sugars (ug g^{-1} fresh fruit) were quadratic with equation of $Y = -0.142 X^2+0.137 X+3.64$ (R^2 = 0.736) and $Y = -64.26 X^2+1358.0 X+20825.2$ (R^2 = 0.421), respectively.

Spraying with concentration of 10 ml l^{-1} RRW also tend to increase starch content of ripe fruits (Table 4), however the increases were non-significant.

Table 3. The effects of spraying different concentrations of rice rinsed water to leaves of strawberry plants on soluble solid content (Brix), ethanol soluble sugar content* (μ g^{-1} fresh weight (FW) and μg^{-1} dry weight (DW) of ripe fruits (mean of six replicates)

No.	Concentration of rice rinsed water (ml l^{-1})	Soluble solid content (Brix*)	Ethanol soluble sugar content of ripe fruits (μ g^{-1} FW)	Ethanol soluble sugar content of ripe fruits (μ g^{-1} DW)
1.	0	4.6^{c**}	21135^{b}	1094.8^{a***}
2.	5	4.9^{bc}	23723^{b}	1035.3^{a}
3.	10	5.8^{a}	32978^{a}	1592.5^{a}
4.	15	5.3^{abc}	22358^{b}	1031.8^{a}
5.	20	5.3^{ab}	23638^{b}	903.8^{a}

Phenol test [16]; ** Means followed by similar letters indicating non-significant different (P>0.05); * After log transformation*

Table 4. The effects of spraying different concentration of rice rinsed water to leaves of strawberry plants on starch concentrations (μ g^{-1} fresh weight (FW) and μg^{-1} dry weight (DW) of ripe fruits (mean of six replicates)

No.	Concentration of rice rinsed water (ml l^{-1})	Starch content of ripe fruits (μ g^{-1} FW)	Starch content of ripe fruits (μ g^{-1} DW)
1.	0	1554.05^{a*}	47500^{a}
2.	5	1435.72^{a}	43413^{a}
3.	10	2066.09^{a}	52248^{a}
4.	15	2232.68^{a}	49427^{a}
5.	20	1617.51^{a}	50535^{a}

** Means followed by similar letters in the same column are non-significant different (P=0.05)*

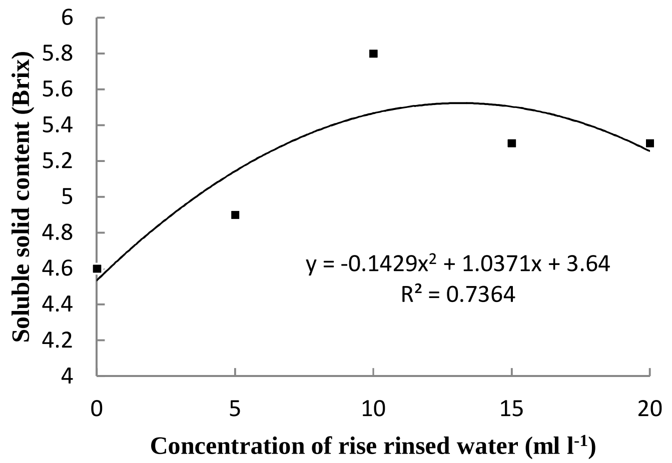

Fig. 1. Relationship between concentrations of rice rinsed water (ml l^{-1}) and soluble solid content (Brix) in fresh fruits

3.3.3 Starch content and the number and weight of fresh fruits

Spraying with 10 ml l^{-1}RRW also tend to increase starch content of ripe fruits (Table 4), the number of young fruits, turning pink fruits, ripe fruits and total number of fruit per plot (Table 5), tend to increase the weight of young fruits, turning pink fruits, ripe fruits and total weight of fruit per plot (Table 6), however the differences were non-significant. Increasing the concentration of spray above 10 ml l^{-1} of RRW generally had no effects.

Table 5. The effects of spraying different concentrations of rice rinsed water to leaves of strawberry on number of young fruits, fruits turning pink and ripe fruits (fruits plot^{-1}) at the end of experiment (means of six replicates)

No.	Concentrations of rice rinsed water (ml l^{-1})	Number of fruits (fruits plot^{-1})			
		Young fruits	Turning pink	Ripe	Total
1.	0	17 ^{a*}	6 ^{a}	7 ^{a}	30 ^{a}
2.	5	22 ^{a}	8 ^{a}	11 ^{a}	41 ^{a}
3.	10	24 ^{a}	8 ^{a}	9 ^{a}	41 ^{a}
4.	15	20 ^{a}	10 ^{a}	10 ^{a}	40 ^{a}
5.	20	16 ^{a}	5 ^{a}	10 ^{a}	31 ^{a}

** Means followed by similar letters in the same column are non-significant different (P=0.05)*

Table 6. The effects of spraying different concentrations of rice rinsed water to leaves of strawberry on the weight of young fruits, fruits turning pink and ripe fruits (g plot^{-1}) at the end of experiment (means of six replicates)

No.	Concentrations of rice rinsed water (ml l^{-1})	Weight of fruits (g plot^{-1})			
		Young fruits	Turning pink	Ripe	Total
1.	0	41.7 ^{a*}	27.5 ^{a}	49.50 ^{a}	118.7 ^{a}
2.	5	45.8 ^{a}	36.8 ^{a}	72.67 ^{a}	115.3 ^{a}
3.	10	47.7 ^{a}	39.0 ^{a}	56.50 ^{a}	143.2 ^{a}
4.	15	39.7 ^{a}	45.7 ^{a}	68.70 ^{a}	154.1 ^{a}
5.	20	31.5 ^{a}	27.8 ^{a}	71.20 ^{a}	130.5 ^{a}

**Means followed by similar letters in the same column are non-significant different (P=0.05)*

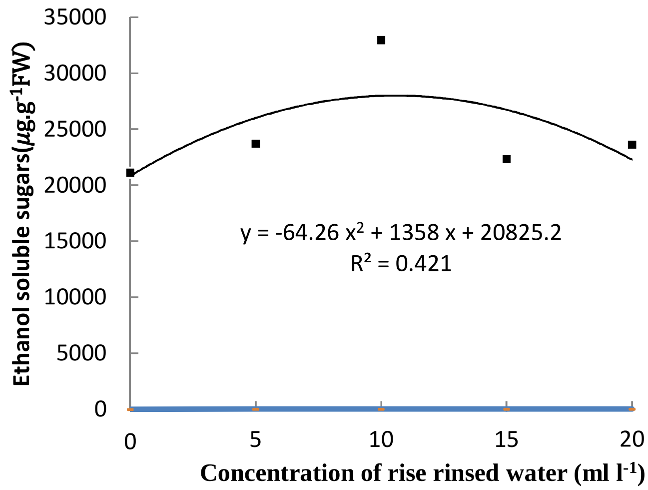

Fig. 2. Relationship between concentrations of rice rinsed water (ml l^{-1}) and ethanol soluble sugars content in fresh fruits (μg g^{-1}FW)

4. DISCUSSION

The lowest soluble sugars were found in Super C4 variety of rice (*Oryza sativa* cv. Super C4) (Table 1) which might be due to genetic factor, shortest rice growing duration or condition during processing of the rice. C4 variety is generally cooked in average Indonesian household, because of the lowest price. C4 rice was chosen for the experiment because of its lower soluble sugar content in anticipation that the soluble sugars if transported passively to the leaves would not decrease appreciably water potential in the cells. The size of starch cells was smaller compared to the size of stomata openings suggesting that starch cells will be able to enter the leaves along with water and passively transported into the leaf cells. In the leaves the starch will be stored temporally in the vacuole during the day, and transformed into sugars and transported to other parts of the plants during the night and used for respiration and other uses [13]

Increasing concentration of RRW sprayed to the leaves up to 10 ml l^{-1} increased SSC (Table 3) and ethanol soluble sugars of ripe fruits (Table 3) suggesting that the sugars were used for fruit filling, further increase generally had no effect. Means minimum and maximum air temperature during the experiment were 15.0℃ and 25.8℃ (Table 2) which were slightly higher than temperature of 25/12°C suggested by Wang and Camp [9] as optimum temperature for highest soluble sugars content of ripe fruits. MacKenzie et al. [10] indicated that at constant temperature of 15℃, ripe strawberry fruits contained 6.5% of RRW, and 5.2% at 25℃. In this experiment the RRW of fruits were 4.6-5.3 which might be caused by higher minimum temperatures or lower incident solar radiation. In higher elevation in the tropics, clouds frequently cover the sky reducing the intensity of solar radiation [5] hence, the rate of photosynthesis, resulting in reduction in transport of sugar to fruits and other parts of the plants. Crop loads as also the age of the strawberry plants, might also had some effects.

Spraying with 10 ml l^{-1}RRW also tends to increase starch content (Table 4), the number (Table 5) and weight of young, mature and ripe fruits (Table 6). The non-significant effects of spaying RRW in this experiment might be due to short duration of the experiment. Acuna-Maldonado and Pritts [11] increased CO_2 content surround the leaves in previous season to increase the carbohydrates reserves in the roots and stem of strawberry plants in the following season and found that in the following season fruit yields increased 21%, and increased sugars and soluble carbohydrates in the leaves. However, the soluble solid and sugar contents in the fruits were not reported. Cordenunsi et al. [19] found that during six days after harvest, the starch in cold storage fruits was changed into sugars, indicating that, in this experiment albeit small differences, higher starch content would eventually increase fruit sugar content.

Quadratic correlations were found between concentration of RRW to soluble solid (Fig. 1) and sugar content (Fig. 2). The use of sugars for other purposes (respiration and transport to other sinks) might cause that the increase in concentration of RRW sprayed had no effects on sugar content of ripe fruits.

Strawberry variety used in this experiment was an everbearing variety in which during fruiting, photosynthates in the form of sugars were also transported to storage organ in roots and stems for the next growth of shoots, flowering and fruit growth and fruit filling. If the transport to storage organs was low, growth of the plant, flowering, fruits growth and fruit filling in the next season will be affected and harvest will be low [19,10], Ruan et al. [20], however, whether the RRW spray will increase the starch in storage organs and how its affect fruit yields and sugar content in the next season need further investigation.

Rice rinsed water, generally found in average Indonesian households during rice cooking in the morning, is usually wasted. With the present population of Indonesia (250 millions) and assuming half of that number cook rice, with consumption of 100 kg person year, RRW (1:2 g/ml) obtained was 25 000 million liters. With RRW containing 778,9 μg ml^{-1}soluble sugars and 1494,7 μg ml^{-1} starch, Indonesian people wasted 19 000 ton of sugars and 37 000 ton of starch per year. Spraying this resources to increase sugars content and yields of fruits is one way to make use of these wasted resources. In addition RRW also contains minute amount of amino acids, vitamins and other substances, the effects of which need further elucidation.

5. CONCLUSION

It is concluded that spraying strawberry leaves with 10 ml l^{-1} rice rinsed water, increased soluble solid and sugar content of ripe fruits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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