Tan, Sing P.; Parks, Sophie E.; Stathopoulos, Costas E.; Roach, Paul D. "Greenhouse-grown bitter melon: production and quality characteristics", Journal of the Science of Food and Agriculture Vol. 94, Issue 9, p. 1896-1903 (2014)

Available from: http://dx.doi.org/10.1002/jsfa.6509

Greenhouse-grown bitter melon: production and quality characteristics

Sing P. Tan[1*], Sophie E. Parks[1,2], Costas E. Stathopoulos[1], Paul D. Roach[1]

[1]School of Environmental and Life Sciences, University of Newcastle, Ourimbah, New South Wales, Australia

[2] NSW Department of Primary Industries, Central Coast Primary Industries Centre, Locked Bag 26, Gosford, New South Wales 2250, Australia

[*]*Corresponding author:* Sing P. Tan

School of Environmental and Life Sciences

University of Newcastle

10 Chittaway Road, Ourimbah

NSW 2258 Australia.

Tel. no.: +61452421121

Fax no.: +61 2 4348 4145

Email Address: SingPei.Tan@newcastle.edu.au

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jsfa.6509

Abstract

BACKGROUND: Bitter melon (*Momordica charantia* L.) is a medicinal fruit reported to have antidiabetic properties. To grow this tropical fruit year-round in temperate climates, greenhouse production is necessary, sometimes without insect pollinators. Suitable high yielding varieties with good bioactivity need to be identified. An experiment evaluated the yield of six varieties of bitter melon under greenhouse conditions and their bioactivity in terms of total phenolic and saponin compounds, and total antioxidant activity determined using four assays.

RESULTS: The larger varieties (Big Top Medium, Hanuman, Jade and White) were more productive than the small varieties (Indra and Niddhi) in terms of total fruit weight and yield per flower pollinated. The bioactivity (total phenolic and saponin compounds and antioxidant activity) of two small varieties and Big Top Medium were significantly higher than the other three large varieties.

CONCLUSION: Preliminary research has identified Big Top Medium as the most suitable variety for greenhouse production. Two antioxidant assays, 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP), were shown to provide the strongest correlations with phenolic and saponin compounds of bitter melon. The rich source of phenolic and saponin compounds and their associated antioxidant activity highlight bitter melon as a valuable food ingredient.

Keywords: Bitter melon; greenhouse; yield; bioactivity; antioxidants

INTRODUCTION

Bitter melon, *Momordica charantia* L. (Cucurbitaceae), is a tropical medicinal vine cultivated for its edible fruit. It is reportedly rich in phenolic and saponin compounds and these are associated with high antioxidant activity.[1, 2] Studies have shown the effectiveness of extracted fresh, juiced or even dried bitter melon in the treatment of diabetic animals and in type 2 diabetic human subjects.[3, 4] Many studies have demonstrated an association between bitter melon fruit and beneficial effects on health including anti-cancer,[5] anti-viral,[6] anti-inflammatory,[7] hypolipidaemic and hypocholesterolaemic effects.[8]

Studies report that the therapeutic effects, claimed as a result of the use of bitter melon in traditional medicine, may be due to the action of these strong antioxidants contained in bitter melon.[9] It is possible to measure the content of phenolic and saponin compounds, and their associated antioxidant activities. In particular, total antioxidant activity (TAA) assays are very useful tools for evaluating the antioxidant activity in foods. There are many assays, such as oxygen radical absorbance capacity (ORAC), 2,2'-azinobis-(3-thylbenzothiozoline-6-sulfonic acid) (ABTS), 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP), which are commonly used to determine the TAA of foods. Each assay type can produce unique set of results due to different test systems.[10] No single assay is adequate for measuring the TAA of foods accurately because each assay type has its advantages and limitations.[11] Therefore, it is recommended at least two assay types are used to evaluate the activity of antioxidant in foods.[12]

Fruits of bitter melon varieties differ in colour, shape and size, and research indicates that varieties may also differ in the contents of their bioactive compounds.[1, 13] However, it remains to be demonstrated whether varieties grown under controlled conditions differ in bioactive characteristics. This needs consideration since the production environment is known to have an important effect on the bioactive constituents of other Cucurbitaceae fruits. For example, increasing fertiliser rate was shown to decrease the antioxidant profile of pumpkin (*Cucurbita pepo*) fruit[14] and organic growing conditions increased the phenolic compound content and antioxidant activity of 10 melon (*Cucumis melo* L.) cultivars.[15]

The greenhouse system is a useful production method for extending the season of tropical plants such as bitter melon in temperate areas. This system also has the benefit of excluding pests, such as the melon fruit fly *Bactrocera cucurbitae*, a widely distributed and major pest of bitter melon in temperate, tropical and sub-tropical regions.[16] However, insect pollinators are also excluded from entering the greenhouse and therefore, assisted pollination is required for bitter melon fruit set in greenhouse systems. Hand pollination has been shown to be as effective as bee pollination for bitter melon.[17] It represents a substantial financial cost of production and therefore, obtaining a high yield for each pollinated flower is a desirable agronomic trait. In addition to the yield, it is also important to determine the quality of fruits in terms of their bioactivity.

To our best knowledge, studies identifying high yielding bitter melon varieties for greenhouses, particularly those without insect pollinators, have not been reported. Furthermore, the bioactivity of bitter melon could be a valuable marketable trait used to differentiate bitter melon varieties but bioactivity has not been well described for varieties

grown under the same conditions. This study aimed to evaluate the whole fruits of bitter melon varieties based on their bioactive qualities and agronomic traits for greenhouse production.

MATERIALS AND METHODS

Chemicals

Methanol was obtained from Merck Pty. Ltd. (Victoria, Australia). Folin-Ciocalteau (FC) reagent, sodium carbonate, ABTS, potassium persulfate, DPPH, fluorescein, potassium phosphate, vanillin, sodium acetate trihydrate, acetic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric (III) chloride hexahydrate, sulphuric acid, gallic acid, aecsin and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Pty. Ltd. (Castle Hill, Australia). The 2, 2'- azobis (2 - amidinopropane) dihydrochloride (AAPH) was purchased from Wako Pure Chemical Industries, Ltd, Osaka, Japan. Deionised water was prepared on the day of use with a Milli-Q Direct 16 water purification system (Millipore Australia Pty Ltd, North Ryde, Australia).

Greenhouse production of bitter melon

The experiment was conducted at the NSW Department of Primary Industries research station in Narara, NSW, Australia (151^{o} 19' E, 33^{o} 23' S). Seeds of six bitter melon varieties were included in the experiment. Seeds of three varieties: Big Top Medium, White F1 (referred to here as White) and Jade F1 (referred to here as Jade) were obtained from Asian-Seed (New Zealand), and seeds of three varieties: Hanuman F1-277 (referred to here as Hanuman), Niddhi 393 (referred to here as Niddhi), and Indra were obtained from East-West Seeds International (Thailand). The variety Hanuman represents the

common type of bitter melon grown in Australia. The seeds were sown in rockwool blocks and placed in a propagation house with an average temperature of 25 ^{o}C and average relative humidity of 80%. When the seedlings had two true leaves they were transferred to one of four climate-controlled replicate greenhouses and planted into bags of coir in a run-to-waste hydroponic system. The plants were fertigated with a complete nutrient solution with a target electrical conductivity (EC) of 1.2 dSm^{-1} and pH of 5.0-6.5. The temperature in each of the four greenhouses was maintained between 18 and 30 ^{o}C and the relative humidity was maintained between 60 and 80%.

The six varieties were arranged in a randomised block design with four replicates, one replicate allocated to each greenhouse. Plants were spaced along the two centre rows of the greenhouse and were trained up and outwards from the centre on wire trellises. An experimental unit was limited to a single plant of each variety due to space and labour constraints. Once flowering commenced, all receptive female flowers were hand pollinated with pollen from male flowers of the same variety. This simulated a greenhouse system without insect pollinators. Pollinated flowers were tagged for monitoring.

Yield measurements

At each harvest, the total number and total weight of all marketable fruits from each plant was recorded. For a subsample (90% of the total) of fruit, the length, circumference, weight, and time from pollination to harvest was recorded for each fruit. The amount of fruit produced per flower pollinated was used to indicate the productivity of each variety. Bitter melon total fruit weights and numbers were expressed on a per plant basis. Fruit

age at harvest was the time taken for fruit to develop between pollination and harvest of the marketable fruit and was expressed on a per plant basis.

Fruit set was expressed as the number of marketable fruits produced as a percentage of the number of flowers pollinated per plant. Fruit weight was also expressed as marketable fruit weight obtained per flower pollinated, per plant.

Drying of bitter melon

During fruit production, three batches of six fresh whole fruits from each of the six varieties of bitter melons were randomly selected and rinsed with deionised water and dried thoroughly with a clean paper towel and stored at -20 ºC before vacuum oven drying. Each batch formed a replicate. For drying, each batch of bitter melon was cut into slices of approximately 1 to 2 mm thickness and placed in an aluminium tray, weighed and then dried in a vacuum oven at 65 °C and -70 kPa for 48 h (Thermoline, Australasian Scientific Marketing Group, Australia Scientific, Australia).

After vacuum oven-drying, the six fruit from each of the three batches for the six varieties were weighed before being combined and then ground into powder using a commercial blender (Waring, John Morris Scientific, Australia). The three replicate batches of powders from each of the six varieties were kept in sealed containers before analysed were conducted and refrigerated.

Preparation of extracts from bitter melon powders

The bitter melon powders were extracted by adding 1 g of powder to 100 mL of deionised water and heating the solution using a shaking water bath at 80 °C for 1 h. Triplicate samples of each of the three replicate batches of powders from the six varieties were extracted. Each of the 18 experimental runs for this part of the experiment consisted of 3

samples. After extraction, the samples were allowed to cool down and settle for 10 min on ice. The extracts were centrifuged at 6,000 × g for 10 min at 10 ºC (Beckman J2-MC Centrifuge, JA-20, The Spinco Business Center of Beckman Intruments, INC., USA) before the supernatant from each sample was filtered through a 0.45 μm syringe filter (Phenomenex, Pennants Hills, Australia). A total 18 runs was conducted in random order.

Analytical analysis

All measurements were performed in triplicate, with the mean of the 3 samples for each experimental run used in the subsequent statistical analysis.

Total phenolic compounds

The total phenolic compounds (TPC) of samples were determined according to Cicco et al.[18] with some modifications. Briefly, 200 μL of diluted bitter melon extracts, standard solution and blank were pipetted into separate test tubes. 200 μL of FC reagent was added into each test tube. The solution was mixed well and allowed to stand for 2 min to equilibrate. Then, 1600 μL of a 5% sodium carbonate solution was added to each tube. The solutions were mixed using a vortex mixer and placed in the dark at room temperature for 2 h. The absorption of the solution was measured at 765 nm using a spectrophotometer (Carry 50 Bio, Varian Pty. Ltd., Australia). Gallic acid was used as a standard and TPC were expressed as gallic acid equivalent (GAE), mg GAE kg^{-1} on a wet basis and mg GAE g^{-1} on a dry basis.

Total saponin compounds

The total saponin compounds (TSC) of samples was analysed using the method of Hiai et al.[19] with some modifications. In brief, 300 μL of the diluted sample was mixed with 300

μL of 8% (w/v) vanillin solution and 3 mL of 72% (v/v) of sulphuric acid. The sample was mixed and incubated at 60 ˚C for 15 min and then cooled on ice for 10 min. The absorption of the solution was measured at 560 nm using the spectrophotometer. Aecsin was used as a standard and TSC was expressed as aecsin equivalent (AE), mg AE kg^{-1} on a wet basis and mg AE g^{-1} on a dry basis.

ORAC assay

The TAA of the bitter melon extracts was determined according to the method of Price, Sanny and Shevlin[20] with some modifications. The ORAC assay was conducted using a FLUOstar Omega microplate reader (BMG LABTECH Pty. Ltd., Mount Eliza, Australia). In brief, 10 nM fluorescein and 240 mM AAPH solutions were heated to 37 °C before adding to the 96 well microtitre plate. The plate was preincubated at 37 °C for at least 10 min before the addition of the AAPH to allow the plate to equilibrate to 37 °C. To measure the ORAC value, 10 mM potassium phosphate buffer (pH 7.4), Trolox (25, 12.5 and 6.25 μM), and gallic acid (20 and 10 μM) were used as a blank, standard and control, respectively. Then, 25 μL sample or standard or control was transferred to the microtitre plate followed by the addition of 25 μL of AAPH and 150 μL fluorescein solutions into each. The ORAC values of the samples were calculated on the basis of a Trolox standard curve and the TAA was expressed as Trolox equivalent (TE), μmol TE g^{-1} of dry basis.

ABTS assay

The ABTS assay was conducted according to Thaipong et al.[21] with some modifications. Stock solutions of 7.4 mM ABTS and 2.6 mM potassium persulfate were prepared and

kept at 4 ˚C until use. The working solution was prepared by mixing the two stock solutions in equal quantities and incubating them for 12 to 16 h in the dark at room temperature (20 ˚C). Then, 1 mL of the working solution was mixed with 60 mL methanol to obtain an absorbance of 1.1±0.02 units at 734 nm measured using the spectrophotometer. A fresh working solution was prepared for each assay. The diluted sample (150 µL) was mixed with 2850 µL of the working solution and incubated for 2 h in the dark at room temperature. The absorption of the solution at 734 nm was measured using the spectrophotometer. Trolox was used as a standard and TAA was expressed as Trolox equivalent (TE), µmol TE g^{-1} of dry basis.

DPPH assay

The TAA of BM extract was measured using the method described by Kubola and Siriamornpun[22] with some modifications. In brief, a stock solution of 0.6 M DPPH was prepared and kept at -20 ˚C until use. The working solution was prepared by mixing 10 mL of stock solution with 45 mL of methanol to obtain an absorbance of 1.1±0.02 units at 515 nm measured using the spectrophotometer. The diluted sample (150 µL) was mixed with 2850 µL of working solution. The sample was allowed to stand for 30 min. The absorption of the solution was measured at 515 nm using the spectrophotometer. Trolox was used as a standard and TAA was expressed as Trolox equivalent (TE), µmol TE g^{-1} of dry basis.

FRAP assay

The FRAP assay was measured according to Thaipong et al.[21] with some modifications. The stock solutions of 300 mM acetate buffer (3.1 g sodium acetate trihydrate and 16 mL acetic acid, pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM ferric (III) chloride

hexahydrate in deionised water were prepared and kept at 4 ˚C until use. The fresh working solution was prepared by mixing 100 mL of acetate buffer, 10 mL of TPTZ and 10 mL of ferric (III) chloride hexahydrate in a ratio of 10:1:1. The working solution was then incubated at 37 ˚C before use. The diluted sample (150 µL) was mixed with 2850 µL of the working solution for 30 min in the dark at room temperature. The absorption of the solution was measured at 593 nm using the spectrophotometer. Trolox was used as a standard and TAA was expressed as Trolox equivalence (TE), µmol TE g^{-1} of dry basis.

Statistical analyses

Results were presented as mean values with standard deviations. The bitter melon growth and quality responses were tested using analysis of variance (ANOVA) with means compared using the Bonferroni post-hoc test at a 5% significance level. Correlations among data obtained were calculated using Pearson's correlation coefficient (r). The SPSS software version 19.0 statistical package (IBM Corp., United States) was used for all analyses.

RESULTS

Bitter melon growth responses

Yield measurements

Individual fruit lengths and weights, and fruit age (days from pollination to harvest), provide an indication of the size of the fruit varieties used in the experiment (Table 1). Individual fruits of Indra and Niddhi were considerably lighter in weight and shorter in

length than Big Top Medium, Hanuman, Jade and White (Table 1). Consequently, Indra and Niddhi were categorised as small fruit and Big Top Medium, Hanuman, Jade and White were categorised as large fruit varieties.

The varieties with the smallest fruits (Indra and Niddi) produced the least total fruit weight per plant and the lowest yield per flower pollinated (Table 2). Their fruits also took the least time to develop from pollination to harvest (Table 1). Indra was the more productive variety of the two, producing significantly more fruits than any other variety and the highest fruit set success (Table 2). It also had an earlier production peak over 6 week the harvest period (Figure 1). The total fruit weight for six bitter melon varieties over the harvest periods, indicating production peak, for 6 weeks is shown in Figure 1.

Of the four larger varieties, Big Top Medium produced the highest yield as total fruit weight, significantly more than White but not Hanuman or Jade. It also appeared to achieve peak production earlier than the other larger varieties (Figure 1). Big Top Medium had a higher fruit set than the other large fruit varieties, except for Hanuman. Jade, with the largest fruit size produced the most yield per flower pollinated but was found non-significant with Big Top Medium.

Of the four larger varieties, Big Top Medium produced the highest yield as total fruit weight, significantly more than White but not Hanuman or Jade. Big Top Medium also appeared to achieve peak production earlier than the other larger varieties (Figure 1). Jade, with the largest fruit size produced the most yield per flower pollinated and had a higher fruit set than the other large fruit varieties.

Bitter melon fruit quality responses

Total phenolic compounds

The TPC of six varieties of bitter melon on a wet and dry basis is shown in Table 3. Generally, the TPC of the smaller fruit varieties (Indra and Niddhi) were higher than the larger varieties on a wet and dry basis. Indra had the highest TPC followed by Niddhi, and Big Top Medium on a dry basis. Big Top Medium had significantly more TPC than the other large fruit varieties (Hanuman, Jade and White) on a dry basis but no significant difference on a wet basis was observed.

Total saponin compounds

The TSC of six varieties of bitter melon was presented on a wet and dry basis (Table 3). The TSC of the smaller fruit varieties (Indra and Niddhi) were generally higher than the larger varieties. Niddhi and Indra had the highest TSC followed by Big Top Medium on a wet and dry basis. However, the difference in TSC between Indra and Big Top Medium on a dry basis was found to be non-significant. Jade and White had the lowest TSC among six varieties (dry basis). The TSC (wet basis) of Jade was lower than Hanuman and White but no significant difference between Jade and White was found.

Total antioxidant activity

The TAA measured in water extract, obtained using ORAC, ABTS, DPPH and FRAP assays from six varieties of bitter melon powders differed among the six varieties of bitter melon but generally Big Top Medium had the highest TAA of the six varieties (Figure 2). For the ORAC measurement (Figure 2a), Big Top Medium had significantly more TAA than all other fruit varieties. Figure 2a shows Big Top Medium had the highest TAA whereas White had the lowest TAA among the six varieties. The TAA of Indra, Niddhi,

Hanuman and Jade was not significantly different from each other (Figure 2a). For ABTS (Figure 2b), DPPH (Figure 2c) and FRAP (Figure 2d), the TAA of Big Top Medium was lower than Indra and Niddhi, but not significantly lower.

Relationship between phenolic and saponin compounds with the antioxidant activity

The TPC (dry basis) of bitter melon (Table 3) was strongly correlated with TAA obtained from ABTS, DPPH and FRAP assays. However, a poor correlation between TAA and ORAC was found. The TSC (dry basis) of bitter melon (Table 3) was also positively associated with TAA (Figure 2) obtained from ORAC, ABTS, DPPH and FRAP assays.

DISCUSSION

Bitter melon growth responses

Greenhouse production has the advantage of prolonging the season in a temperate region and in excluding pests. We successfully demonstrated a greenhouse bitter melon crop using assisted pollination. This preliminary study has shown that in terms of yield, Jade and Big Top Medium performed as well as, or better than, the standard bitter melon type (Hanuman) and thus are good alternative varieties. The large fruit variety White had a lower yield compared with the other large fruits which perhaps reflected a later peak in fruit production. In eggplant, varieties exhibiting earliness were associated with higher yields.[23] The yield of the two small varieties (Indra and Niddhi) was limited compared with the larger varieties making them less desirable for greenhouse production. Although Indra produced the largest number of fruits per plant, this did not compensate for the small size of the fruit and limited total weight of fruit produced. As yield data was limited in this study, future research is necessary to estimate the potential yield of these varieties under commercial production.

Natural pollinators are excluded from the greenhouse so assisted pollination is required for bitter melon fruit set and this represents an additional economic cost. Further, in this study, fruit set was generally low. Poor pollination technique may have been a factor since in another study hand pollination was as effective as bee pollination for bitter melon (var. Galaxy).[17] It is also possible that some plants were self-pollinated in this study since the male flowers were collected and pooled but were not labelled prior to being used for pollination. The occurrence of self-pollination would have reduced the number of fruits set in the case of these varieties not having self-compatibility. Self-compatibility was not evaluated in this study but it is a reproductive process for other cucurbit species. In any case, pollination techniques would need to be optimised for commercial production of bitter melon to maximise fruit set in greenhouses.

Bitter melon fruit quality responses

This study has demonstrated that the bitter melon varieties differ significantly in their levels of bioactive content and TAA when grown under the same conditions. To our knowledge, this has not been demonstrated previously. Estimating the bioactivity and antioxidant potential (TPC, TSC and TAA) of six varieties of bitter melon has highlighted the generally greater bioactivity and antioxidant potential of varieties with smaller sized fruits.

Total phenolic compounds

The two small varieties had the highest TPC (Table 3) but since the yield of these varieties was limited (Table 2), they may be less suitable as greenhouse varieties.

However, Big Top Medium would be a more suitable choice as a greenhouse variety. It had the highest TPC among the four large fruit varieties and its TPC was only about 20% lower than that determined for the smaller varieties.

The production of phenolic compounds in bitter melon fruits is potentially a response to stressful conditions.[1] Therefore, it might be expected that the controlled greenhouse environments used in this study were limiting to the accumulation of phenolic compounds. However, the TPC of bitter melon in this study ranged from 5.1 to 7.9 mg GAE g^{-1} of dry basis (Table 3) and were similar to those obtained in another study from four varieties of bitter melon (6.7-8.0 mg GAE g^{-1} of dry basis).[1] Furthermore, the TPC of the bitter melon grown in this study were much higher than those grown in Thailand (3.2 mg GAE $g^{-1}$of dry basis).[22] In a study on Pak Choi, phenolic contents were not significantly affected by greenhouse and field environments.[24] Nonetheless, the dependency of bioactive contents in bitter melon on environmental factors requires further investigation since anecdotally we have observed higher levels of TPC in another bitter melon crop produced in the field (data not shown).

Total saponin compounds

The antidiabetic properties of bitter melon are reportedly linked to its bioactive compounds including saponins.[2, 4] Many individual saponin compounds of bitter melon have been isolated and identified[25] but the data in relation to the TSC of bitter melon is currently limited. In the present study, six varieties of bitter melon had a TSC, ranging from 46.8 to 93.2 mg AE g^{-1} of dry basis (Table 3), which is higher than the TSC reported for a number of Chinese medicinal plants.[26, 27] Being rich in saponins, bitter melon has the potential to be used as active ingredient in pharmaceutical foods.

Total antioxidant activity

The present study demonstrated that the six varieties of bitter melon possess potent antioxidant activity measured using four assays (ORAC, ABTS, DPPH and FRAP). The antioxidant activity depended on the assay type as each assay employed a unique test system and produced free radicals using different processes.[12] Similar trends in the TAA of the assays for the six bitter melon varieties suggest that several assays can be used to evaluate antioxidant activity of these fruits. However, there are several limitations for each antioxidant assay type.[11] To measure the TAA of bitter melon, DPPH and FRAP assays were shown to provide the strongest correlations with the bioactive components (phenolics and saponins) (Table 4). In another bitter melon study, [22] a strong positive relationship was also demonstrated between TPC and these assays.

In general, the TAA of the smaller bitter melon varieties (Indra and Niddhi) was higher than larger varieties (Figure 2). This may be due to a higher ratio of skin to flesh for the smaller bitter melon varieties resulting in a higher and denser bioactivity per gram of fruit. Smaller Winesap apple fruits were shown to be richer in vitamin C because the skin contained more vitamin C than the pulp and the ratio of skin to pulp was higher.[28] However, further studies are needed to verify this for bitter melon.

The bitter melon grown in this study had a generally good source of TAA compared with the values published for other vegetables. Levels were similar to two other Cucurbitaceae vegetable fruits, cucumber and squash, and 12 other vegetable types but were lower than 8 vegetable types.[29] Contrasting results are published for broccoli. Bitter melon had a lower TAA than that reported for broccoli in one study but a higher TAA than reported in

another study. [29, 30] This may reflect the different sources of broccoli used between the studies since they were probably grown and stored under contrasting conditions, affecting their TAA. Similarly, methods of analyses may affect TAA. For example, the bitter melon grown in this study had a higher TAA (Figure 2d), than fourteen other varieties of bitter melon.[31] The difference may have arisen from the use of different fruit parts for the analyses as the flesh of the fourteen varieties was used to determine TAA, whereas the TAA of whole fruit, including flesh and seed, was evaluated in the present study. It is also important to be aware that TAA measured in bitter melon and other vegetables and fruits can be affected by extraction solvent, [30, 32] vegetable maturity stage [33] and environmental conditions.[34]

CONCLUSIONS

For greenhouse production, Big Top Medium was the most suitable variety of the six studied based on its combined high yield and high bioactivity. However, the two small varieties (Indra and Niddhi) exhibited the highest phenolic (7.9 and 7.5 mg GAE g^{-1} dry basis, respectively) and saponin (89.3 and 93.2 mg AE g^{-1} dry basis, respectively) compounds and TAA, perhaps based on their higher skin to pulp weight ratio. The comparison of the bioactive contents and antioxidant activity of bitter melon with reported values for other vegetables suggests bitter melon has potential as a high-value ingredient for incorporation into pharmaceutical foods.

ACKNOWLEDGEMENTS

The authors acknowledge the University of Newcastle, Australia, for the financial support through a PhD grant for Sing Tan and would like to thank Lorraine Spohr for reviewing the manuscript, and Carly Murray, Basem Al-Khawaldeh and Joshua Jarvis for their excellent technical assistance in producing the bitter melon crop. This study was also funded as part of an Australian Centre for International Agricultural Research project.

REFERENCES

Horax R, Hettiarachchy N and Islam S, Total Phenolic Contents and Phenolic Acid Constituents in 4 Varieties of Bitter Melons (*Momordica charantia*) and Antioxidant Activities of their Extracts. *J Food Sci* **70**:C275-C280 (2005).

Keller AC, Ma J, Kavalier A, He K, Brillantes AB and Kennelly EJ, Saponins from the traditional medicinal plant *Momordica charantia* stimulate insulin secretion in *vitro*. *Phytomedicine* **19**:32-37 (2011).

Zänker KS, Mang B, Wolters M and Hahn A, Personalized diabetes and cancer medicine: A rationale for anti-diabetic nutrition (Bitter Melon) in a supportive setting. *Curr Cancer Ther Rev* **8**:66-77 (2012).

Clouatre DL, Rao SN and Preuss HG, Bitter melon extracts in diabetic and normal rats favorably influence blood glucose and blood pressure regulation. *J Med Food* **14**:1496-1504 (2011).

Yasui Y, Hosokawa M, Sahara T, Suzuki R, Ohgiya S, Kohno H, Tanaka T and Miyashita K, Bitter gourd seed fatty acid rich in 9c,11t,13t-conjugated linolenic acid induces apoptosis and up-regulates the GADD45, p53 and PPARgamma in human colon cancer Caco-2 cells. *PLEFA* **73**:113-119 (2005).

Beloin N, Gbeassor M, Akpagana K, Hudson J, de Soussa K, Koumaglo K and Arnason JT, Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. *J Ethnopharmacol* **96**:49-55 (2005).

Hsu C, Tsai T-H, Li Y-Y, Wu W-H, Huang C-J and Tsai P-J, Wild bitter melon (*Momordica charantia* Linn. var. *abbreviata* Ser.) extract and its bioactive components suppress *Propionibacterium acnes*-induced inflammation. *Food Chem* **135**:976-984 (2012).

Nerurkar PV, Lee YK and Nerurkar VR, *Momordica charantia* (Bitter Melon) inhibits primary human adipocyte differentiation by modulating adipogenic genes. *BMC Complem Altern M* **10**:34 (2010).

Wu SJ and Ng LT, Antioxidant and free radical scavenging activities of wild bitter melon (*Momordica charantia* Linn. var. abbreviata Ser.) in Taiwan. *LWT-Food Sci Technol* **41**:323-330 (2008).

Kelebek H, Jourdes M, Selli S and Teissedre P-L, Comparative evaluation of the phenolic content and antioxidant capacity of sun-dried raisins. *J Sci Food Agric* **93**:2963-2972 (2013).

Moore J and Yu L, Methods for Antioxidant Capacity Estimation of Wheat and Wheat-Based Food Products, in *Wheat Antioxidants*. John Wiley & Sons, Inc., pp 118-172 (2007).

Moon J-K and Shibamoto T, Antioxidant Assays for Plant and Food Components. *J Agric Food Chem* **57**:1655-1666 (2009).

Habicht SD, Kind V, Rudloff S, Borsch C, Mueller AS, Pallauf J, Yang RY and Krawinkel MB, Quantification of antidiabetic extracts and compounds in bitter gourd varieties. *Food Chem* **126**:172-176 (2011).

Oloyede FM, Agbaje GO, Obuotor EM and Obisesan IO, Nutritional and antioxidant profiles of pumpkin (*Cucurbita pepo* Linn.) immature and mature fruits as influenced by NPK fertilizer. *Food Chem* **135**:460-463 (2012).

Salandanan K, Bunning M, Stonaker F, Külen O, Kendall P and Stushnoff C, Comparative analysis of antioxidant properties and fruit quality attributes of organically and conventionally grown melons (*Cucumis melo* L.). *HortScience* **44**:1825-1832 (2009).

Dhillon MK, Singh R, Naresh JS and Sharma HC, The melon fruit fly, Bactrocera cucurbitae: A review of its biology and management. *J Insect Sci* **5** (2005).

Deyto RC and Cervancia CR, Floral biology and pollination of ampalaya (*Momordica charantia* L.). *Philipp Agric Sci* **92**:8-18 (2009).

Cicco N, Lanorte MT, Paraggio M, Viggiano M and Lattanzio V, A reproducible, rapid and inexpensive Folin-Ciocalteu micro-method in determining phenolics of plant methanol extracts. *Microchem J* **91**:107-110 (2009).

Hiai S, Oura H and Nakajima T, Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. *Planta Med* **29**:116-122 (1976).

Price JA, Sanny CG and Shevlin D, Application of manual assessment of oxygen radical absorbent capacity (ORAC) for use in high throughput assay of "total" antioxidant activity of drugs and natural products. *J Pharmacol Toxicol Methods* **54**:56-61 (2006).

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L and Byrne DH, Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J Food Compos Anal* **19**:669-675 (2006).

Kubola J and Siriamornpun S, Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts in vitro. *Food Chem* **110**:881-890 (2008).

Muñoz-Falcón JE, Prohens J, Vilanova S and Nuez F, Characterization, diversity, and relationships of the Spanish striped (Listada) eggplants: A model for the enhancement and protection of local heirlooms. *Euphytica* **164**:405-419 (2008).

Zhao X, Nechols JR, Williams KA, Wang W and Carey EE, Comparison of phenolic acids in organically and conventionally grown pac choi (*Brassica rapa* L. chinensis). *J Sci Food Agric* **89**:940-946 (2009).

Raman A and Lau C, Anti-diabetic properties and phytochemistry of *Momordica charanti*a L. (Cucurbitaceae). *Phytomedicine* **2**:349-362 (1996).

Chen Y, Roan H, Lii C, Huang Y and Wang T, Relationship between antioxidant and antiglycation ability of saponins, polyphenols, and polysaccharides in Chinese herbal medicines used to treat diabetes. *J Med Plant Res* **5**:2322-2331 (2011).

Vuong QV, Hirun S, Roach PD, Bowyer MC, Phillips PA and Scarlett CJ, Effect of extraction conditions on total phenolic compounds and antioxidant activities of *Carica* papaya leaf aqueous extracts. *J Herb Med* (2013).

Batchelder EL and Overholser EL, Factors affecting the vitamin C content of apples. *J Agric Res* **53**:547-551 (1936).

Cao G, Sofic E and Prior RL, Antioxidant Capacity of Tea and Common Vegetables. *J Agric Food Chem* **44**:3426-3431 (1996).

Sun T, Powers JR and Tang J, Evaluation of the antioxidant activity of asparagus, broccoli and their juices. *Food Chem* **105**:101-106 (2007).

Huang L., Deng Yuan-Yuan, Zhang Ming-Wei, Zhang Y., Wei Zhen-Cheng, Zhang Rui-Fen and Xiao-Jun T, Phenolic Profiles and Antioxidant Activity of *Momordica charantia* L. of Different Varieties. *China Agr Sci* **44**:4660-4668 (2011).

Sun T and Ho CT, Antioxidant activities of buckwheat extracts. *Food Chem* **90**:743-749 (2005).

Horax R, Hettiarachchy N and Chen P, Extraction, Quantification, and Antioxidant Activities of Phenolics from Pericarp and Seeds of Bitter Melons (*Momordica charantia*) Harvested at Three Maturity Stages (Immature, Mature, and Ripe). *J Agric Food Chem* **58**:4428-4433 (2010).

Zhao X, Iwamoto T and Carey EE, Antioxidant capacity of leafy vegetables as affected by high tunnel environment, fertilisation and growth stage. *J Sci Food Agric* **87**:2692-2699 (2007).

Table 1. Morphological characteristics of fruits (marketable) for six bitter melon varieties, hand pollinated and grown in climate controlled greenhouses

Variety	Individual fruit weight (g)	Individual fruit length (mm)	Number of samples	Fruit age (days from pollination to harvest)	Number of samples
Big Top Medium	340.0±64.7^{c}	149.7±16.3^{c}	83	18.0±0.7^{a}	79
Indra	43.0±17.8^{d}	108.1±22.3^{d}	140	8.9±1.2^{c}	115
Niddhi	40.0±16.7^{d}	87.8±16.6^{e}	56	9.2±0.6^{c}	52
Hanuman	345.4±113.3^{c}	260.5±34.2^{b}	58	12.2±1.2^{b}	55
Jade	481.7±119.3^{a}	331.0±47.0^{a}	40	14.6±1.9^{b}	35
White	413.6±153.8^{b}	245.6±42.3^{b}	26	14.2±1.3^{b}	26

Values for individual fruit weight and fruit length are means of measured fruits ± standard deviation. The fruit age was not recorded for every fruit.

Table 2. Total yields of marketable fruits for six bitter melon varieties, hand pollinated and grown in climate controlled greenhouses

Variety	Total fruit weight (g)	Total fruit number	Fruit set success (%)	Yield/ pollinated flower (g)
Big Top Medium	7055.9±104 2.8[a]	20.8±2.8[b]	43.3±3.8[a b]	165.3±37. 9[ab]
Indra	1504.6±351. 0[bc]	35.0±8.1[a]	58.5±2.9[a]	25.8±6.0[c]
Niddhi	560.5±295.0 [c]	14.0±7.1[bc]	33.5±12. 7[bc]	17.1±5.8[c]
Hanuman	5195.1±142 5.8[ab]	15.3±4.6[bc]	35.3±10. 1[bc]	148.5±12. 6[b]
Jade	4817.0±356 0.4[ab]	10.0±7.2[bc]	18.3±11. 7[c]	246.3±61. 9[a]
White	2688.1±116 2.1[bc]	6.5±2.4[c]	17.5±6.5[c]	152.6±38. 8[b]

Values for total fruit weight, total fruit number, fruit set and yield per pollinated flower are means per plant ± standard deviation (n=4).

Table 3. Total phenolic and saponin compounds for six bitter melon varieties

Varieties	Total phenolic compounds		Total saponin compounds	
	mg GAE kg^{-1}, wet basis	mg GAE g^{-1}, dry basis	mg AE kg^{-1}, wet basis	mg AE g^{-1}, dry basis
Big Top Medium	411.4±34.3 ^{b}	6.2±0.2 ^{c}	5259.7±26 6.9 ^{b}	79.3±1.9 ^{b}
Indra	678.0±67.5 ^{a}	7.9±0.1 ^{a}	7618.4±57 6.7 ^{a}	89.3±2.2 ^{ab}
Niddhi	600.4±24.6 ^{a}	7.5±0.2 ^{b}	7475.1±82. 4 ^{a}	93.2±4.0 ^{a}
Hanuman	338.5±41.5 ^{b}	5.1±0.2 ^{e}	4509.0±83 4.9 ^{bc}	68.3±7.3 ^{b}
Jade	329.8±18.1 ^{b}	5.2±0.2 ^{de}	2965.0±28 5.9 ^{d}	46.8±1.4 ^{c}
White	340.4±20.8 ^{b}	5.6±0.1 ^{d}	3406.1±14 8.3 ^{cd}	56.0±1.9 ^{c}

Values (means of measured fruits ± standard deviation) in a column not sharing a superscript letter are significantly different from each other (P <0.05).

Table 4. Correlation between phenolic and saponin compounds with the antioxidant activity

	TPC	TSC
ORAC	0.256	0.446
ABTS	0.713**	0.678**
DPPH	0.885**	0.860**
FRAP	0.859**	0.859**

**. Correlation is significant at the 0.01 level (2-tailed).

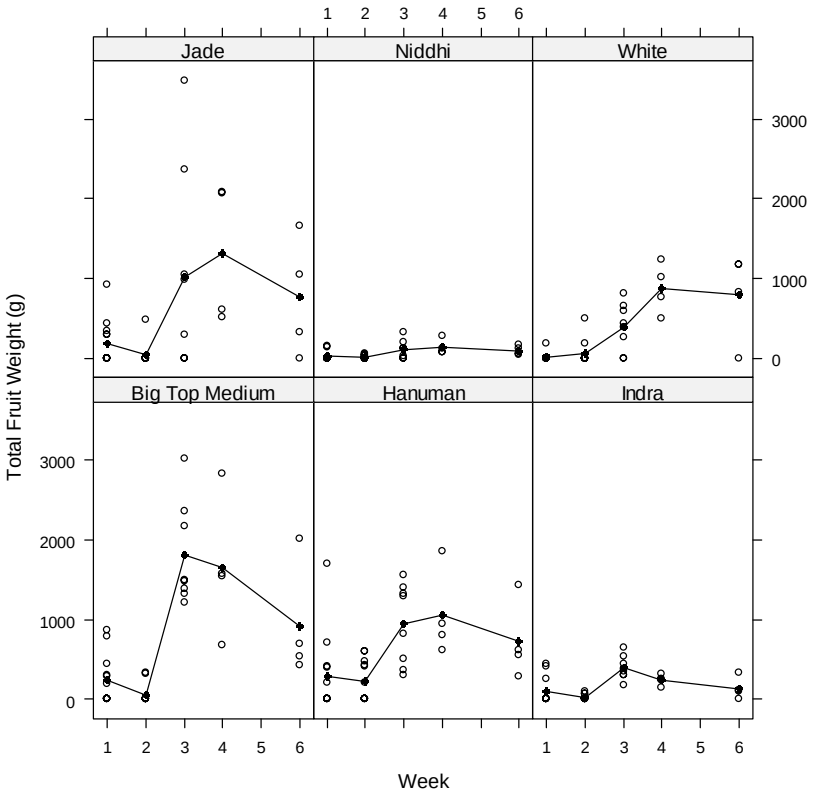

Figure 1. Weekly total fruit weight (g) for six bitter melon varieties

Open circles are raw data, filled circles are means. In some weeks, several harvests were taken (10 in total over 6 weeks).

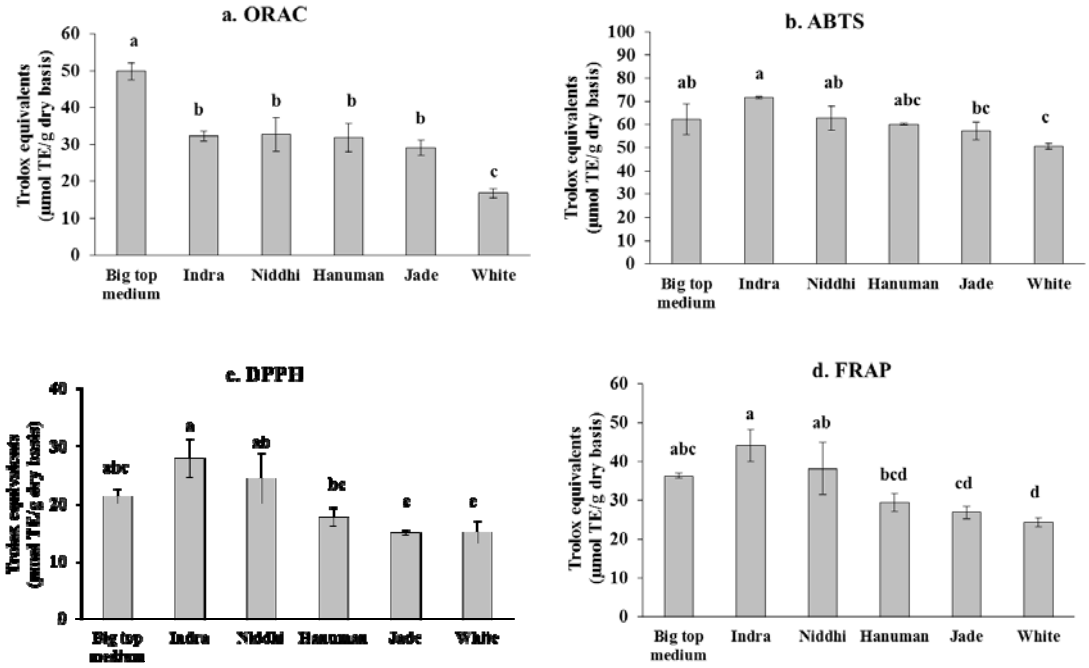

Figure 2. The total antioxidant activity of six varieties of bitter melon obtained from ORAC (a), ABTS (b), DPPH (c) and FRAP (d) assays. *Values* (*means of measured fruits ± standard deviation) not sharing a superscript letter are significantly different from each other (P < 0.05).*