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Research Article

Customized Cooking Methods Enhance Antioxidant, Antiglycemic, and Insulin-Like Properties of *Momordica charantia* and *Moringa oleifera*

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The current study compares antioxidant activities, total phenolic content (TPC), vitamin C content, and antiglycemic properties of *Momordica charantia* (small bitter gourd) and *Moringa oleifera* (drumstick leaves) before and after subjecting to boiling and microwave heating for different durations. Both cooking methods enhanced the antioxidant activity and vitamin C content in the vegetables studied when cooked for five minutes and these properties declined when the cooking time was prolonged to 20 minutes. Cooking also retained or slightly improved the α-glucosidase enzyme inhibition activity of the vegetables; however, it reduced the ability of the vegetable extracts to inhibit α-amylase enzyme activity. The antioxidant activities were positively correlated with the TPC and vitamin C content in the vegetable extracts tested. The present study also evaluated the insulin-like properties (stimulation of adipogenesis) of selected vegetable extracts (five minutes microwaved). 3T3-L1 adipocytes treated with small bitter gourd extract significantly stimulated lipogenesis (in the absence of insulin) compared to drumstick leaves. Thus, the finding of this study negates the belief that cooking will reduce the nutritional value of the vegetables and also suggested that appropriate cooking method and duration for different vegetables could be selected to improve or preserve their nutritional value.

1. Introduction

Oxidative stress is a consequence due to imbalance of the reactive oxygen species (ROS) production and elimination, where it can be synthesized from endogenous and exogenous sources. Carcinogenesis which includes damage to lipids and nucleic acids has been associated with excess production of ROS [1]. The etiology of numerous other diseases such as diabetes, cardiovascular, and Alzheimer's is also linked to free radicals and oxidative stress [2]. Increased intake of antioxidants from food is encouraged for healthy lifestyle and antioxidants from dietary sources have drawn the public's interest. Fruits and vegetables play an important role in a healthy diet. Adequate intake of fruits and vegetables rich in antioxidant content is important for prevention of degenerative diseases that are caused by oxidative stress [3]. Most vegetables are cooked before consumption and the cooking process had been shown to affect the medicinal properties of the vegetables [4–6].

Several studies have shown that different cooking methods can improve the antioxidant capacity of some vegetables [4, 7], while other studies have shown that vegetables are best eaten raw to preserve thier antioxidant properties which are lowered when they are cooked [8–10]. However, a fixed conclusion regarding the ideal cooking method and duration that generally yields the highest antioxidant could not be derived. There are many ways of cooking and some researchers suggested that there should be a balanced intake of raw and cooked vegetables [11] while others proposed that every vegetable could be chosen to a preferred cooking method to preserve or enhance its nutritional qualities and values [12, 13]. Some researchers claimed that prolonged cooking time can also cause a greater loss of antioxidant capacity [9] while others showed improved antioxidant capacity in some of the selected vegetables [12].

3T3-L1 preadipocyte cell line is proven to be an excellent and cost effective model for preliminary screening of various

bioactive compounds as potential antidiabetic and antiobesity agents, particularly glucose metabolism, as these cells can differentiate from preadipocyte fibroblastic form to adipocyte under appropriate culture conditions [14]. 3T3-L1 cells have a fibroblast-like morphology but under appropriate conditions, the cells differentiate into mature adipocyte-like phenotype. Since dietary management is a starting point for the treatment of DM and obesity, it is pertinent to investigate the effects of vegetable extracts on proliferation and 3T3-L1 preadipocytes differentiation into matured adipocytes.

In the present study, the vegetables with medicinal properties were selected. Bitter gourd *(Momordica charantia)* is one of the commonly consumed vegetables among Malaysians. It has a variety of uses as a traditional herbal medicine worldwide including potential to treat HIV infection [15]. In Indian Ayurvedic medicine, bitter gourd is often seen as an "insulin plant" and is therefore highly recommended for diabetics. In some countries, it has been used with great success to treat diabetes and control blood sugar [16]. Bitterness of this melon comes from the high content of quinine and coincidentally, was regarded by Asians, as an agent for preventing and treating malaria [17]. It is considered as emetic, diuretic, stimulant, stomachic, tonic, and also valuable in treating gout, disease of liver and spleen, and rheumatism. It has moreover been demonstrated to have hypoglycemic properties (antidiabetic) in animal model and also human studies [18]. Similarly, *Moringa oleifera* is also a highly valued plant which is mostly distributed in the tropical and subtropical countries. A variety of bioactive compounds with important pharmacological/nutraceutical properties have been isolated from different parts of this plant such as the leaves (excellent source of protein, vitamins, and minerals), seeds and immature pods (good source of oleic acid), roots, bark, fruit, and flowers [19]. *Moringa oleifera* is used as a traditional medicine, particularly in South Asia for its various therapeutic effects [20].

Commonly, these two plants/vegetables are cooked by boiling, microwaving, or deep- or stir-frying prior to consumption. However, the effect on antioxidant capacity, vitamin C content, total phenolic content, as well as antiglycemic ability of these selected local vegetables subjected to common styles of cooking and different durations of cooking is yet to be addressed. Thus, the present study endeavors to focus on the optimum cooking system (technique and duration) that results in the highest retention of the antioxidant capacity and radical scavenging activities, antiglycemic properties, and insulin-like activity of small bitter gourd *(Momordica charantia)* and drumstick leaves *(Moringa oleifera)*.

2. Material and Methods

2.1. Chemicals. All chemicals used in the present study were of analytical grade. Phosphate-buffered saline, gallic acid ($C_7H_6O_5$), ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), L-ascorbic acid (L-AA), L-ascorbate oxidase from *Cucurbita* sp., 6-hydroxy-2,5,7,8-tetramethyl-chroman-2 carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine and 2,2-diphenyl-1-picrylhyrazyl (DPPH), α-Glucosidase *(Saccharomyces cerevisiae)*, α-amylase (porcine pancreatic Type IV-B), DL-dithiothreitol (DTT), 4-nitrophenyl-α-D-glucopyranoside (PNPG), 3,5-dinitrosalicylic acid (DNS), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), 0.25% trypsin EDTA, penicillin/streptomycin mixture, 1-methyl-3-isobutylxanthine (IBMX), 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Oil Red O dye, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Folin–Ciocalteu's reagent, glacial acetic acid, hydrochloric acid (fuming) (HCl), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), potassium persulfate ($K_2O_8S_2$), sodium acetate trihydrate, and sodium carbonate (Na_2CO_3) were obtained from Merck® (Darmstadt, Germany). Absolute ethanol and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased, respectively, from Fisher Scientific® UK Ltd. (Loughborough, Leicestershire, UK) and Boehringer Mannheim GmbH® (Penzberg, Baden-Wurttemberg, Germany). Ultra pure water was obtained from a water filtration system by Millipore® Co. (Billerica, Massachusetts, USA).

2.2. Vegetables and Processing. Small bitter gourd *(Momordica charantia)* was purchased from local market at Pantai Dalam, Kuala Lumpur, Malaysia, and drumstick leaves *(Moringa oleifera)* were obtained from Puchong, Selangor, Malaysia. Four hundred grams of each vegetable were washed and cleaned thoroughly and the excess water was dab-dried with paper towel before weighing. The vegetables were divided into 50 g portions and subjected to the following cooking methods:

(i) *Raw*: vegetables were placed in a beaker containing 250 mL of distilled water and immediately processed for juice extraction.

(ii) *Boiling*: three portions of each vegetable were added into separate beakers, each containing 250 mL distilled water placed on a hot plate and boiled for three different durations which were 5, 10, and 20 minutes.

(iii) *Microwaving*: three portions of each vegetable were added into microwave tupperware, each containing 250 mL of water, and cooked in a commercial-1000 W microwave oven at high power for three different durations which were 5, 10, and 20 minutes.

The cooked vegetables and the remaining liquid were cooled rapidly on ice to prevent further cooking from residual heat and then homogenized with an electric blender. The homogenate was then filtered using a nylon mesh and centrifuged at 2,400 ×g for 20 minutes to obtain a clear supernatant which was then freeze-dried. The lyophilized aqueous extracts were refrigerated at 4°C and were reconstituted with water on the day of experiment.

2.3. Antioxidant Assays

2.3.1. Diphenyl-1-picryl-hydrazyl (DPPH) Radical Scavenging Assay. This assay was conducted according to the method outlined by Tan et al. [13]. Ascorbic acid (0–1000 μM) was used as standard and the free radical scavenging activity was expressed as percentage of DPPH quenched.

2.3.2. Trolox Equivalent Antioxidant Capacity (TEAC) Assay. This assay was performed according to the method by Ng et al. [12]. Trolox (0–250 μg/mL) was used as standard and the antioxidant capacity was expressed as mmol trolox equivalent in 100 g lyophilized extract (mmol TE/100 g).

2.3.3. Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP assay was carried out according to the method by Benzie and Strain [21]. Iron (II) sulfate heptahydrate (0–1000 μM) was used as the standard. The FRAP values were expressed as mmol iron sulfate equivalent in 100 g lyophilized extract (mmol FE/100 g).

2.3.4. Measurement of Vitamin C Content. FRASC assay is a modification of FRAP assay for the measurement of vitamin C (ascorbic acid). The vitamin C content in the vegetable extracts was determined based on the method adapted from Szeto et al. [22] with slight modification. The FRAP reagent was prepared prior to the experiment. One hundred μL of vegetable aqueous extracts (5 mg/mL) was mixed with 40 μL of ascorbate oxidase solution (5 IU/mL). Another set of 100 μL of extracts was mixed with 40 μL of distilled water. Both (with ascorbate oxidase and without ascorbate oxidase) assay mixtures were incubated at 37°C for 5 minutes. The aliquots were then cooled immediately on ice. Once cooled, ten μL of the extracts was loaded into the wells and 300 μL of freshly prepared FRAP reagent was added. Using a microplate reader, the absorbance was measured after four minutes at 593 nm. All samples were analyzed in triplicate assays. Ascorbic acid (0–150 μg/mL) was used as standard. Preparation of the standard was done by using ascorbate oxidase and water (two sets) similarly to the sample preparation. The vitamin C content was expressed as mg ascorbic acid equivalent in 100 g lyophilized extract (mg AE/100 g).

2.3.5. Determination of Total Polyphenols Content (TPC). The TPC in the vegetable extracts were determined according to the method outlined by Oki et al. [23]. Gallic acid (0–100 μg/mL) was used as standard and the total phenolic content was expressed as mg gallic acid equivalent in 100 g lyophilized extract (mg GAE/100 g).

2.4. Antiglycemic Assays (α-Amylase and α-Glucosidase Enzyme Inhibitory Activity). The enzyme inhibition assays were performed according to Manaharan et al. [24]. Acarbose was used as positive control for both assays. The percentage of inhibition of the samples was calculated according to the following formula:

$$\text{Percentage of inhibition (\%)} = \frac{\text{Control}_A - \text{Sample}_A}{\text{Control}_A} \times 100, \quad (1)$$

where A = absorbance.

2.5. Cell Culture and Differentiation. 3T3-L1 mouse preadipocytes were cultured in DMEM supplemented with 10% (v/v) heat-inactivated FBS at 37°C in a humidified atmosphere of 5% CO_2. Lipogenesis was induced in confluent 3T3-L1 preadipocyte culture by incubating in differentiation medium 1 (DM1) which contains 0.5 mM IBMX, 1 μM dexamethasone, and 10 μg/mL insulin in growth media for 48 hours. Then, the medium was changed to differentiation media 2 (DM2) which contains vegetable aqueous extracts at various concentrations (0.5–100 μg/mL) and incubated for another 48 hours [14]. As for the basal control, ultra pure water was used instead of aqueous extract. The medium was changed every 2-3 days and replaced with fresh growth media. All experiments, unless otherwise indicated, were performed in triplicate.

2.5.1. MTT Colorimetric Assay. The effect of vegetable aqueous extracts on the viability of 3T3-L1 preadipocyte was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay [25]. Preadipocytes were seeded at a density of 2000 cells per well in 96-well tissue culture plate and were allowed to attach for 24 hours prior to the proliferation assay. Subsequently, the preadipocytes were treated with the aqueous extracts at different concentrations and incubated for 48 hours before MTT reagent was added into each well. As for the basal control, ultra pure water was used instead of the aqueous extracts. After four hours, the culture medium containing MTT was carefully removed. Subsequently, 100 μL of DMSO was then added into each well and left on a plate shaker at room temperature for five minutes to make sure all formazan crystals were dissolved completely. The absorbance of formazan was measured at 560 nm.

2.5.2. Oil Red O Quantification Assay. 3T3-L1 adipocytes were fixed in 0.5% (v/v) paraformaldehyde after various treatments and stained with freshly prepared 0.5% (v/v) Oil Red O for 15 minutes as described previously by Subramaniam et al. [14]. For quantitative analysis of Oil Red O retention in these cells, stained adipocytes were extracted with absolute isopropanol and absorbance was measured at 510 nm. Results were expressed in percentage of difference as compared to untreated cells.

2.6. Statistical Analysis. All experiments were performed in triplicate and at least three separate experiments were conducted to ensure reproducibility. The data shown are single representation of three separate experiments. Values are expressed as mean ± standard deviation (unless otherwise stated) calculated using Microsoft Excel 2013 or SPSS version 16. Statistical significance was accepted at a level of $p \leq 0.05$ and $p \leq 0.001$.

3. Results and Discussion

Generally, the effect of cooking method and duration of the two vegetables tested (drumstick leaves and bitter gourd) showed some differences in the antioxidant and antiglycemic properties and vitamin C content. In the DPPH radical scavenging assay, boiling and microwaving significantly ($p \leq 0.05$) enhanced the radical scavenging ability of both

vegetables studied, and especially drumstick leaves showed 10-fold increases after cooking regardless of cooking durations (Table 1). Ng et al. [12] and Tan et al. [13] reported that both boiled and microwaved samples showed increased DPPH radical scavenging activity, which is in agreement with the results from present study. The increase in radical scavenging potency after cooking was presumably due to the alteration of the oxidation state of polyphenols by heating whereby the intermediate oxidation state of polyphenols possessed higher radical scavenging ability compared to the nonoxidised one [26]. Moreover, some heat-stable antioxidant compounds such as carotenoid, which acted as potent radical scavenger, were not affected during the cooking process [26, 27]. However, the small bitter gourd showed a slight decrease in scavenging activity when boiling was prolonged to 20 minutes (Table 1). In this assay, ascorbic acid was used as the experimental positive control and it scavenged 50% of the DPPH radical at 15 μmol (data not shown) compared with 21.6 μmol [12].

Similar to DPPH assay, TEAC assay also measures the ability of antioxidants in vegetable aqueous extracts to scavenge synthetic ABTS radicals. The reason why both DPPH and TEAC assays were carried out was because DPPH assay has its limitation due to colour interference and solubility. Thus, TEAC assay using ABTS radicals was done for further confirmation of the radical scavenging activity because ABTS radical is a moderately stable radical species compared to DPPH radical. Moreover, both hydrophilic and hydrophobic antioxidants can be accessed and the spectral interference is lower when using TEAC assay [28]. Boiling and microwaving for 20 minutes significantly decreased ($p \leq 0.05$) the antioxidant activity in all small bitter gourd compared to 5 and 10 minutes' cooking durations (Table 1). Further, drumstick leaves demonstrated higher antioxidant capacity than small bitter gourd, regardless of cooking method and durations. Similar to DPPH assay, TEAC values of uncooked (raw) vegetables were 3- to 4-fold lower than the cooked vegetables (Table 1).

On the whole, the FRAP level of both vegetables was significantly ($p \leq 0.05$) improved after subjecting to cooking process regardless of the cooking method and duration (Table 1). Moreover, the FRAP level between boiled and microwaved vegetable extracts were insignificant. As for the raw samples, small bitter gourd and drumstick leaves showed almost comparable FRAP levels. However after cooking, the FRAP levels in drumstick leaves elevated more than in small bitter gourd. Interestingly, the increase of this activity was accompanied by the increase of total phenolic content, thus suggesting that the antioxidant activity in drumstick leaves was contributed mainly by phenolic compounds (Table 1).

FRASC assay is a modification of FRAP assay for the measurement of vitamin C. The principle behind this assay is similar to FRAP assay which estimates the total antioxidant capacity of a specimen. As expected, the lowest vitamin C content was detected in uncooked small bitter gourd and drumstick leaves, which were 13.2 mg and 4.6 mg per 100 g lyophilized extract, respectively. Cooked samples showed significant ($p \leq 0.05$) increase of vitamin C content in both vegetables. For drumstick leaves, five minutes microwaved sample showed highest vitamin C content (85.5 mg/100 g lyophilized extract) and the second highest was shown by five minutes' boiled samples (74.5 mg/100 g lyophilized extract). For small bitter gourd, microwaved samples showed no significant difference between the durations of cooking while ten minutes' boiled samples showed highest vitamin C content (60.6 mg/100 g lyophilized extract). Prolonged cooking (20 minutes) showed decreased vitamin C content in both cooking methods but was higher than the respective raw samples. The increase in vitamin C content in the cooked vegetables could be attributed to the increased extractability of ascorbic acid from the vegetable extracts due to the matrix softening by heat rather than the thermal degradation during the cooking process [12]. Moreover, many published findings had reported that vitamin C is destroyed during cooking as it is not stable at high temperature [7, 28]. The heat stress (thermal treatments) may affect the vitamin C content in the extracts by altering the stability and functionality of the endogenous enzymatic protein (ascorbic acid oxidase, AAO) in the vegetables [29]. One of the strategies to prevent the loss of vitamin C is to inactivate the endogenous AAO enzyme. Depending on the thermal stability of AAO and heating durations/conditions, the enzyme could be either partially or completely denatured. Thus, the lower time of cooking could explain the lower losses of ascorbic acid content observed in both the vegetables.

Total phenolic content (TPC) in both vegetables showed an increase when boiled and microwaved. The increase in the total phenolic compounds in cooked samples may be because of the intense breakdown of cell walls and release of phenolic compounds caught in the fiber of vegetables [28]. However, similar to DPPH, FRAP, and TEAC assay, drumstick leaves showed higher TPC level compared to small bitter gourd in both cooked and raw samples. Small bitter gourd showed highest increase in TPC level when microwaved and boiled up to ten minutes. When cooked for 20 minutes, it showed significant ($p \leq 0.05$) decrease of TPC levels in both cooking methods but was higher than its raw sample (Table 1), whereas drumstick leaves did not show any significant changes when cooked using different methods and durations but were higher than their raw extract (Table 1). This result concurs with Turkmen et al. [4] who also reported that there were increased antioxidant values in some of the vegetables after cooking using microwave, boil, and steam methods.

The decreased levels of radical scavenging activity, total phenolic contents, and iron reducing ability after prolonged cooking durations could be due to prolonged exposure to heat treatment which eventually could disrupt the cell walls and cause more phenolic compounds to breakdown [30]. Prolonged exposure to heat treatment might lead to degradation of bioactive compounds which were released after short heat treatment [5]. There were also decreased antioxidant values of red pepper when subjected to prolonged cooking method which was up to 15 minutes [31] suggesting that prolonged cooking may not be a good method to obtain the best antioxidant values in cooked vegetables. In the present study, 5 minutes of cooking (both microwave and boil) showed the best levels of antioxidant values and this concurs with Turkmen et al. [4] who also suggested that moderate heat

Table 1: Antioxidant activities, total phenolic content, vitamin C content, and antiglycemic activities of *Momordica charantia* and *Moringa oleifera*.

Vegetable/processing	Antioxidant activities					Antiglycemic activities	
	DPPH (%)	TEAC (mmol TE/100 g)	FRAP (mmol FE/100 g)	FRASC (mg AE/100 g)	TPC (mg GAE/100 g)	α-Amylase (%)	α-Glucosidase (%)
Momordica charantia (small bitter gourd)							
Raw	13.22 ± 1.03^{a}	3.20 ± 0.13^{a}	8.46 ± 0.67^{a}	13.19 ± 0.45^{a}	0.55 ± 0.01^{a}	5.68 ± 15.52^{a}	21.04 ± 0.73^{a}
Boiled							
5 minutes	51.62 ± 1.46^{b}	11.29 ± 2.13^{b}	26.14 ± 1.13^{bc}	41.43 ± 2.41^{bc}	0.83 ± 0.03^{b}	4.46 ± 5.08^{a}	14.83 ± 2.71^{b}
10 minutes	54.24 ± 5.14^{b}	9.91 ± 0.48^{bc}	30.99 ± 6.18^{b}	50.92 ± 3.26^{b}	0.88 ± 0.06^{b}	ND	12.54 ± 1.37^{b}
20 minutes	45.01 ± 1.03^{b}	9.29 ± 0.05^{bc}	22.63 ± 0.61^{c}	37.62 ± 1.73^{c}	0.75 ± 0.03^{bc}	ND	6.64 ± 4.68^{c}
Microwaved							
5 minutes	54.49 ± 0.82^{b}	11.58 ± 2.06^{b}	27.10 ± 1.23^{bc}	44.73 ± 1.52^{bc}	0.81 ± 0.03^{b}	28.22 ± 4.79^{b}	11.12 ± 2.51^{bc}
10 minutes	51.25 ± 1.54^{b}	11.28 ± 0.48^{b}	26.98 ± 1.75^{bc}	43.42 ± 1.31^{bc}	0.83 ± 0.02^{b}	21.60 ± 8.18^{b}	17.45 ± 5.97^{ab}
20 minutes	36.78 ± 2.16^{c}	8.91 ± 1.16^{c}	18.59 ± 0.31^{d}	45.81 ± 1.85^{bc}	0.69 ± 0.02^{c}	10.80 ± 5.61^{bc}	16.03 ± 1.81^{ab}
Moringa oleifera (drumstick leaves)							
Raw	8.85 ± 0.41^{a}	4.40 ± 0.43^{a}	8.04 ± 0.14^{a}	4.60 ± 1.01^{a}	0.68 ± 0.04^{a}	22.03 ± 1.42^{a}	15.82 ± 2.07^{a}
Boiled							
5 minutes	80.79 ± 0.62^{b}	14.54 ± 0.13^{b}	48.92 ± 2.68^{bc}	74.51 ± 4.86^{bc}	1.12 ± 0.04^{b}	10.32 ± 3.41^{bc}	20.51 ± 4.17^{b}
10 minutes	80.67 ± 1.65^{b}	14.69 ± 0.05^{b}	52.52 ± 1.27^{b}	60.59 ± 4.54^{c}	1.16 ± 0.06^{b}	16.97 ± 8.65^{b}	19.25 ± 2.55^{b}
20 minutes	82.79 ± 0.62^{b}	14.65 ± 0.02^{b}	43.72 ± 1.84^{c}	43.54 ± 4.09^{d}	1.15 ± 0.04^{b}	11.62 ± 9.91^{bc}	13.04 ± 2.06^{a}
Microwaved							
5 minutes	81.17 ± 1.23^{b}	13.78 ± 0.48^{b}	54.65 ± 3.71^{b}	85.54 ± 1.04^{b}	1.12 ± 0.07^{b}	14.81 ± 3.93^{b}	13.93 ± 2.94^{a}
10 minutes	80.23 ± 2.36^{b}	14.01 ± 0.58^{b}	47.78 ± 1.97^{bc}	42.00 ± 0.71^{d}	1.04 ± 0.11^{b}	0.21 ± 8.49^{c}	14.83 ± 1.19^{a}
20 minutes	84.29 ± 0.41^{b}	14.62 ± 0.09^{b}	45.36 ± 1.75^{c}	62.89 ± 6.61^{c}	1.13 ± 0.03^{b}	0.19 ± 6.58^{c}	21.56 ± 1.94^{b}

Values are expressed as mean ± standard deviation ($N = 3$). For each vegetable, values within the same column followed by different superscript alphabets are significantly different ($p \leq 0.05$). For the DPPH assay, the IC50 of ascorbic acid standard = 15 μM. For the α-amylase and α-glucosidase assay, the IC50 of acarbose standard = 3.5 μg/mL and 3 mg/mL. ND: Not detected.

Table 2: Correlation analysis between various antioxidant parameters and antiglycemic activities.

Assay	R^2	r	Significance
Between TPC and antioxidant activities			
TPC and FRAP	0.938	0.968	***
TPC and TEAC	0.859	0.927	***
TPC and DPPH	0.914	0.956	***
TPC and FRASC	0.955	0.911	***
Between reduction power and radical scavenging activity			
FRAP and TEAC	0.864	0.929	***
FRAP and DPPH	0.941	0.970	***
Between the two radical scavenging activities			
TEAC and DPPH	0.959	0.979	***
Between TPC assay and antiglycemic assays			
TPC and α-glucosidase inhibition	0.032	0.180	—
TPC and α-amylase inhibition	0.001	0.035	—

The R^2 value denotes the regression value and the r value denotes Pearson's correlation value. The level of significance was expressed as $^{***}(p \leq 0.001)$.

treatment might be useful in improving antioxidant quality of vegetables.

For antiglycemic assays, raw small bitter gourd showed highest inhibition of α-glucosidase enzyme activity compared to the cooked ones (Table 1). For cooked samples, the order of decreasing percentage of α-glucosidase inhibition was as follows: 10 minutes microwave > 20 minutes microwave > 5 minutes boil > 10 minutes boil > 5 minutes microwave > 20 minutes boil. Microwaved small bitter gourd showed the best ability to inhibit α-glucosidase enzyme activity at 10 minutes (17.5%), while boiled samples showed at five minutes (14.8%). For drumstick leaves, the highest percentage (21.6%) of enzyme inhibition was observed when it was microwaved for 20 minutes. There was significant ($p \leq 0.05$) increase compared to five minutes microwaved samples. When boiled for five minutes, it showed highest percentage (20.5%) of α-glucosidase inhibition. However, prolonged boiling caused significant ($p \leq 0.05$) decrease where 20 minutes boiled samples showed the lowest percentage (13.0%) of α-glucosidase inhibition. This might be due to degradation of bioactive compounds which are responsible for the enzyme inhibition activity. Cooking did not destroy the antiglycemic properties in which α-glucosidase enzyme inhibition activity was retained or improved slightly. This will be favorable to the attenuation of postprandial blood glucose spike.

Conversely, the cooked vegetables showed different pattern of inhibition on α-amylase (another carbohydrate digesting enzyme in the gut) activity compared to α-glucosidase. All the boiled small bitter gourd aqueous extracts showed a lowest percentage of α-amylase inhibition compared to its raw and microwaved extracts. Small bitter gourd that was microwaved for five minutes showed the highest percentage (28.2%) of α-amylase inhibition and prolonged microwave cooking up to 10 minutes and 20 minutes showed decrease in enzyme inhibition. For drumstick leaves, raw samples showed highest percentage (22.0%) of α-amylase inhibition compared to cooked ones. The microwaved samples showed significant ($p \leq 0.05$) decline in enzyme inhibition activity over time. The drumstick leaves lost their α-amylase inhibition ability when microwaved up to 10 minutes and 20 minutes (Table 1).

Table 2 shows the regression and correlation analysis between the antioxidant assays, total phenolic content, vitamin C content, and antiglycemic assays. Based on Table 2, it is clear that TEAC level significantly ($p \leq 0.001$) correlated with DPPH radical scavenging potency ($r = 0.979$) in the vegetables aqueous extracts. This result was reasonable as both TEAC and DPPH radical scavenging assays used similar principle in estimation of antioxidant activity in vegetables based on the radical scavenging activity. The difference between the results of these two assays could be due to the different types of free radical generated in the assays [27] as well as their chemical reactivity towards different antioxidant compounds in the vegetable extracts. Besides, TEAC assay measured both the activity of lipophilic and hydrophilic antioxidant in the vegetable extracts whereas DPPH radical scavenging assay measures only the activity of water-soluble antioxidants and TEAC measures the polar and nonpolar antioxidants [12].

The TPC level also significantly ($p \leq 0.001$) correlated with FRAP level ($r = 0.968$), TEAC level ($r = 0.927$), and DPPH radical scavenging activity ($r = 0.956$) in the vegetables aqueous extracts. Since FRAP assay measures the ability of antioxidant to reduce ferric ion, the antioxidant might be one of the phenolic contents that exist in vegetable aqueous extracts, which supports the correlation result between TPC and FRAP assay. Phenolic compounds such as flavonoids were shown as potent radical scavengers in previous published study [26, 32]. From this correlation result, it is pertinent to suggest that phenolic compounds were the main contributors that increased the total antioxidant activity and ferric reducing power in the vegetables extracts. Correlation analyses were also done between TPC and antiglycemic assays. There were no significant correlation found between TPC assay and antiglycemic assays. This is because most of enzyme inhibition activity decreased when the

vegetable aqueous extracts were subjected to cooking. This finding suggests that phenolic compounds and vitamin C do not contribute to antiglycemic properties of the vegetables used in this study.

MTT is a yellow coloured tetrazolium salt which will be reduced by viable cells to form insoluble purple formazan crystals. This is an approach to measure the number of viable cells in each culture well [25]. Since the five minutes' microwaved vegetable extracts showed best antioxidant and antiglycemic activities, these extracts were selected for the subsequent cell based study. The 3T3-L1 preadipocyte was treated with the extracts at various concentrations (0–100 μg/mL) and the cell viability was measured using the MTT assay. There were no significant differences observed in the preadipocytes viability at extract concentration up to 100 μg/mL relative to the control (data not shown). It was therefore decided that the noncytotoxic concentrations of 0–100 μg/mL of the vegetable aqueous extracts were used in the preadipocyte differentiation experiments.

Insulin is capable of inducing lipogenesis, inhibiting lipolysis, and stimulating uptake of glucose and free fatty acids by peripheral cells such as liver, muscle, and adipose tissues [33]. These actions of insulin on lipogenesis and antilipolysis in human adipose tissue in vivo could be physiologically counteracted by β-adrenoceptor agonists such as epinephrine and isoproterenol [34]. Therefore, crude extracts or compounds which are able to stimulate lipogenesis and inhibit adrenaline induced lipolysis as well as enhance uptake of glucose and free fatty acids are known to have "insulin-like" potential and may have the potential as antidiabetic agents. Besides that, antidiabetic drugs such as troglitazone and rosiglitazone have also been reported to enhance preadipocyte differentiation and glucose uptake in adipocyte [35, 36].

Although the defined stage at which preadipocyte can be considered as fully differentiated is not clearly known, differentiated adipocyte can be evidenced by visualizing the lipid accumulation in adipocyte cytoplasm [37]. In the present study, Oil Red O quantification assay was carried out to identify the lipogenic effect of vegetable aqueous extracts (five minutes microwaved) on 3T3-L1 preadipocytes. Highly differentiated adipocytes have more lipid accumulation in the cytoplasm compared to the undifferentiated adipocytes. In order to measure the level of lipogenesis, lipid globules accumulated in adipocytes were stained with Oil Red O dye. Oil Red O dye stained lipids were clearly visible as red globules under an inverted microscope. The results of Oil Red O quantification assay indicated that drumstick leaves aqueous extract stimulated lipogenesis dose-dependently and vice-versa for small bitter gourd where increase in extract concentration showed reduction in differentiation (Figure 1). The small bitter gourd extract exerted the highest lipogenic activity which was approximately 70% increase in lipogenesis compared to experimental control (cells treated with ultra pure water) at a concentration of 0.5 μg/mL. However, when the extract concentration was increased, the small bitter gourd extract showed up to 50% reduction in lipogenesis at its highest concentration tested (100 μg/mL). The experimental positive control, insulin, at 2.5 μg/mL and rosiglitazone, 20 μM (commercial drug) stimulated lipogenesis in adipocytes by 113% and 91%, respectively, when compared to the experimental control.

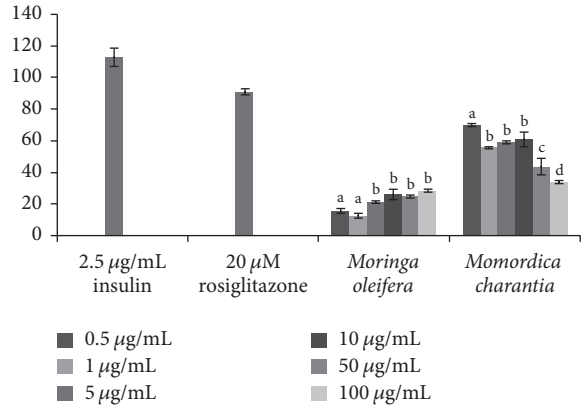

Figure 1: Effect of Insulin and aqueous extracts of *Momordica charantia* and *Moringa oleifera* on lipogenesis in 3T3-L1 preadipocyte. y-axis indicates the mean percentages of lipogenesis ± SD of triplicate assays compared to control values (cells treated with ultra pure water). Preadipocyte (20 000 cells/well, in a 24-well plate) were induced to differentiate with various concentrations of aqueous extracts (5 minutes microwaved) of *Momordica charantia* and *Moringa oleifera* prior to Oil Red O quantification assay. Means with different alphabets within an extract are significantly different ($p \leq 0.05$, ANOVA).

4. Conclusion

The current findings clearly showed that there were differences in nutritional value of cooked and uncooked vegetables. The present findings suggest that short-term cooking increased the nutritional value of the small bitter gourd and drumstick leaves while prolonged cooking reduced some of the antioxidant and antiglycemic properties of the vegetables. Overall, to obtain the best antioxidant and antiglycemic properties, microwaving the small bitter gourd between five to ten minutes will be a better choice. In addition, our study also showed that the small bitter gourd possess notable insulin-like activity compared to drumstick leaves. As for drumstick leaves, five minutes microwaved samples and boiling up to ten minutes showed the best antioxidant value and -glycemic properties. The finding of this study negates the belief that the nutritional value of the cooked vegetables will decrease. This finding would be valuable to nutritionists, food producers, and overall population particularly individuals who are keen to practice healthy diet by consuming vegetables.

Competing Interests

The authors declare that they have no competing interests.

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