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Original research article

Evaluation of Total Phenolic Content, Antioxidant Activity and Anti-Amylase Activity of Different Vegetable and Fruit Mixtures

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ABSTRACT

This work focused on determining the total phenolic content, antioxidant activity, and anti-amylase activity of twenty-one mixtures of vegetable and fruit juices. The results showed that the highest total phenolic content was found in the vegetable and fruit mixture containing guavas (10%), pineapples (10%), ivy gourds (60%), carrots (10%), and Chinese cabbages (10%), while the highest anti-amylase activity was found in the vegetable and fruit mixture containing guavas (10%), pineapples (10%), ivy gourds (50%), carrots (10%), and Chinese cabbages (20%). However, the highest antioxidant activity was found in guava juice. Moreover, the percentages of all vegetable and fruit mixtures, which showed synergistic effects by anti-amylase, total phenolic content, and antioxidant assays, were 81%, 9.5%, and 0%, respectively. For the antagonistic effect, it was only found for 4.8% by total phenolic content assay. Interestingly, a very strong positive correlation between the antioxidant activity and the number of guavas was detected (r = 0.883, p-value = 0.000). Moreover, total phenolic content and the number of carrots showed a weak negative correlation (r = -0.389, p-value = 0.049), while total phenolic content and the number of guavas had a moderate positive correlation (r = 0.424, p-value = 0.031). Therefore, the synergistic formulation may help to manage type 2 diabetes.

Keywords: Vegetable and fruit mixtures; Total phenolic content; Anti-amylase activity; Antioxidant activity

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1. Introduction

Nowadays, the incidence and prevalence of patients with diabetes has exponentially increased in Thailand [1]. Diabetes mellitus is a metabolic syndrome involving a deficiency of insulin secretion or insulin action that leads to high blood glucose levels, and an effect on carbohydrate, fat, and protein metabolism [2-3]. Several factors can contribute to the development of type 2 diabetes, such as consuming unhealthy foods, low education, low awareness, environmental pollutants, low physical activity, and obesity [4]. Increased consumption of high carbohydrate or high sugar foods is one of the main factors that leads to high blood glucose and excess caloric intake. Therefore, decreasing glucose absorption into the bloodstream can cause hypoglycemia which can reduce the risk of developing type 2 diabetes. Alpha-amylase is an important enzyme that digests 1, 4-glucosidic linkages in dietary starch and glycogen to glucose before absorption into the bloodstream. Thus, many studies focus on anti-amylase activity coming from plants, in an effort to control hyperglycemia [5-6].

Moreover, the progression of diabetes mellitus involves oxidative mechanisms [7], which are influenced by many factors, such as lifestyle, age, and environmental pollutants [8-10]. Several studies have reported on the important role antioxidants play in acting against several reactive oxygen species, helping to prevent metabolic syndromes and to stabilize health and various cellular activities in humans [11-12]. Vegetables and fruits are natural sources of vitamins, minerals, phytochemicals, and fiber, which are the main components of healthy foods [13]. Nowadays, consumption of fresh fruit and vegetable juice is rising in popularity throughout the global markets because of their high nutritional value and freshness [14-15].

Several studies have reported that the health benefits of consuming fruit and vegetable juice includes helping to prevent cancers, diabetes, cardiovascular disease, and obesity [13]. Guava (*Psidium guajava* Linn.) is a fruit tree, widely distributed in tropical regions, and is popular in Thailand. Its fruit have high fiber content as well as high amounts of vitamins, minerals, phytochemicals, and low calories, and also has the potential benefit of decreasing blood glucose, serum total cholesterol, triglycerides, LDL, and type 2 diabetic mellitus, however it also can increase HDL levels [16-17]. Pineapple (*Ananas comosus* L. Merr) is a cash crop of Thailand, and is cultivated throughout the country [18]. It has been reported that pineapple is recognized as an intermediate glycemic index (GI) food, a food that can be eaten at a moderate level by diabetic patients that will result in lowering their GI [19]. Ivy gourd (*Coccinia grandis* (L.) Voigt) is an edible medicinal plant, known as leafy vegetable, and has been widely consumed in Thailand for a long time [20]. Previous studies report that the leaves of *C. grandis* have anti-diabetic properties, and can be simply cultivated [21-22]. Carrot (*Daucus carota* subsp. sativus) is a root vegetable, known as a dietary source for α- and β-carotene, precursors to vitamin A in humans [23-24]. Previous studies report that high consumption of β-carotene and α-carotene is correlated with a lower incidence of type 2 diabetes in healthy people [25]. Chinese cabbage (*Brassica rapa* var. Pekinensis) is a cruciferous vegetable popular for cultivation and human consumption. It is a commercial vegetable with a high level of antioxidants, such as flavonoids and phenols [26-27]. Some bioactive compounds, namely myricetin and apigenin, have also been found in this plant [28]. It has been reported that myricetin possesses various beneficial characteristics including hypoglycemic activity, anti-inflammatory activity, anti-oxidative stress, anti-aldose reductase, antinon-enzymatic glycation, and anti-hyperlipidemia [29]. These functions all help to prevent diabetes mellitus and related complications [29].

Formulation of a vegetable and fruit juice mix is a procedure used to improve the nutritional value of the juice mixture, but it depends on plant types used in the procedure [30-31]. A certain combination of fruit and vegetable juices may interact together in an additive, synergistic, or antagonistic manner, depending also on the ingredient ratios and types of the plants. Therefore, the objective of this study was to examine the phenolic content, antioxidant activity, and amylase inhibition of various fruit and vegetable mixtures (guava, pineapple, ivy gourd, carrot, and Chinese cabbage). This analysis and the understanding gained from are vital to the improvement of fruit and vegetable products, pairing ingredients to optimize synergy for the purpose of maximizing their health benefits, especially concerning the treatment and prevention of diabetes mellitus.

2. Materials and Methods

2.1 Materials

Guava (*Psidium guajava* Linn.), pineapple (*Ananas comosus* L. Merr), ivy gourd (*Coccinia grandis* L. Voigt), carrot (*Daucus carota* subsp. sativus), and Chinese cabbage (*Brassica rapa* var. Pekinensis) were purchased from a local market in Ongkarak sub-district, Ongkarak district, Nakhon Nayok, Thailand. The 3,5-dinitrosalicylic acid and acarbose were purchased from Sigma. Sodium potassium tartrate and α-amylase from porcine pancreas were purchased from Sigma-Aldrich and Merck, respectively.

2.2 Plant juice extraction

Guavas, pineapples, ivy gourds, carrots, and Chinese cabbages (discarding the outer leaves of cabbage) were soaked in a 1% sodium bicarbonate solution for 20 min, then soaked in water for 20 min, then washed under running water 3-4 times. The plants were placed in aluminum foil until dry at room temperature [32]. Guavas (PG), pineapple without peel (AC), carrots (DC), leaves of Chinese cabbages (BP), and ivy gourds (CG) were cut into small pieces, then the juice of each plant was extracted by a juice extractor.

2.3 Combination of vegetables and fruits

Each fresh sample of ivy gourds, carrots, Chinese cabbages, guavas, and pineapples was combined according to the ratios listed in Table 1. Then the juice from each plant and combined plants were extracted by juice extractor at medium speed for 2 min. All samples of the extracted juice were kept at 4ºC until analysis.

Table 1. Formulas and ratios for the fruit and vegetable mixtures.

Formula	Ivy gourds (ml)	Carrots (ml)	Chinese-cabbages (ml)	Guavas (ml)	Pineap-ples (ml)
1PACDB	0.6	0.1	0.1	0.1	0.1
2PACDB	0.5	0.1	0.2	0.1	0.1
3PACDB	0.5	0.2	0.1	0.1	0.1
4PACDB	0.4	0.1	0.3	0.1	0.1
5PACDB	0.4	0.2	0.2	0.1	0.1
6PACDB	0.4	0.3	0.1	0.1	0.1
7PACDB	0.3	0.1	0.4	0.1	0.1
8PACDB	0.3	0.2	0.3	0.1	0.1
9PACDB	0.3	0.3	0.2	0.1	0.1
10PACDB	0.3	0.4	0.1	0.1	0.1
11PACDB	0.2	0.1	0.5	0.1	0.1
12PACDB	0.2	0.2	0.4	0.1	0.1
13PACDB	0.2	0.3	0.3	0.1	0.1
14PACDB	0.2	0.4	0.2	0.1	0.1
15PACDB	0.2	0.5	0.1	0.1	0.1
16PACDB	0.1	0.1	0.6	0.1	0.1
17PACDB	0.1	0.2	0.5	0.1	0.1
18PACDB	0.1	0.3	0.4	0.1	0.1
19PACDB	0.1	0.4	0.3	0.1	0.1
20PACDB	0.1	0.5	0.2	0.1	0.1
21PACDB	0.1	0.6	0.1	0.1	0.1

2.4 Total phenolic content

The total phenolic content for each juice mixture was estimated using the Folin-Ciocalteu protocol. Each juice mixture was prepared at dilutions of 1: 2 and 1:10 (300 µl) and then combined with 1.5 mL of

Folin-Ciocalteu reagent and held for 5 min. Then, sodium carbonate (7.5% w/v) was added and held for 30 min at room temperature in the dark. Absorbance was measured at 765 nm (Model T60UV). Each reaction was performed in duplicate. Gallic acid was used as a positive control and to produce a calibration curve. The total phenolic content for each mixture was calculated using the calibration curve and shown as mg/g gallic acid equivalents (GAE) [33-34]. The standard deviation of each sample was calculated from 4 replicate results.

2.5 Free radical scavenging assay

Free radical scavenging activity was estimated using the ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) method. ABTS• + cation radicals were generated by mixing 7 mM ABTS (10 ml) with 140 mM potassium persulfate (179 µl), and left at room temperature in the dark overnight. Before using, the ABTS•+ cation radical solution was diluted with distilled water to provide an absorbance of 0.70±0.01 at 734 nm. Each juice dilution (1:10, 1:50, and 1:100) (20 µl) was mixed with the diluted ABTS• + solution (3.9 ml) and absorbance was measured at 734 nm after incubation for 6 min at room temperature in dark. Each reaction was performed in duplicate. The percentage of free radical scavenging activities was calculated using the formula according to Deetae et al. (2012) and Thummajitsakul et al. (2019) [33-34], finding the effective concentration (EC_{50}) required to inhibit 50% of the free radicals, obtained by linear regression analysis. Thus, a higher EC_{50} value means a lower free radical scavenging capacity. Standard deviation of each sample was calculated from duplicate results.

2.6 Amylase inhibitory activities

Each juice sample dilution was combined with 100 µl of amylase solution (12 units/ml) and incubated at 25°C for 30 min, then a 1% starch solution (100 µl) was added and incubated at 25°C for 30 min. The DNS reagent (100 µl) was mixed in and left at 85°C for 15 min, and then quickly cooled down to 4°C. Each reaction was mixed with distilled water (900 µl), and the absorbance was measured at 540 nm. Each reaction was performed in duplicate, with acarbose used as a positive control. The percentage of amylase inhibition was calculated using a formula according to Thummajitsakul et al. (2019) [34], with inhibition expressed as EC_{50}, indicating the sample concentration required to inhibit 50% of the amylase enzyme present. Standard deviation of each sample was calculated from duplicate results.

2.7 Statistical analysis

Descriptive statistics (i.e., percentage, mean, and SD) were used for total phenolic content, free radical scavenging activity, and amylase inhibitory activity. Analysis of variance (ANOVA) was used to determine differences between the means of the expected and observed values of total phenolic content, free radical scavenging, and anti-amylase activity. Principle component analysis was used to express the correlation between biological effects and the proportion of each kind of fruit and vegetable using Paleontological statistic program version 3.16 [35]. PSPP program version 0.10.5 was used to analyze all statistics [36].

3. Results and Discussion

In the current study, the total phenolic content, free radical scavenging activity, and anti-amylase activity were determined for the juice of ivy gourds, carrots, Chinese cabbages, guavas, and pineapples, and the 21 vegetable and fruit mixtures. The results showed that total phenolic content, free radical scavenging activity, and anti-amylase activity were present in all samples. The total phenolic content, free radical scavenging activity, and anti-amylase activity are listed in Table 2.

Table 2. Total phenolic content, free radical scavenging, and amylase inhibition of each individual vegetable and fruit, and their combinations.

Samples	Total phenolic content (µg gallic acid equivalents/g sample)	Free radical scavenging		Amylase inhibition	
		EC_{50} (%)	$1/EC_{50}$	EC_{50} (%)	$1/EC_{50}$
Guava	1834.5±122.0	0.20±0.00	5.00	0.30±0.03	3.35
Pineapple	929.5±4.1	1.15±0.23	0.87	0.23±0.01	4.36
Ivy Gourd	1200.0±31.0	1.18±0.18	0.85	0.18±0.01	5.49
Carrot	738.0±31.0	2.67±1.70	0.37	0.23±0.03	4.38
Chinese Cabbage	688.3±188.1	2.81±0.89	0.36	1.12±0.07	0.89
1PACDB	2217.5±146.8	1.37±0.43	0.73	0.20±0.02	5.05
2PACDB	1144.4±55.8	1.49±0.17	0.67	0.07±0.01	13.58
3PACDB	916.4±175.7	0.41±0.03	2.44	0.23±0.00	4.42
4PACDB	1239.5±186.1	1.80±0.67	0.56	0.09±0.04	10.58
5PACDB	1277.5±285.3	0.75±0.14	1.33	0.21±0.03	4.87
6PACDB	1014.3±24.8	3.41±0.25	0.29	0.26±0.00	3.89
7PACDB	1125.4±62.0	0.96±0.01	1.04	0.31±1.00	3.26
8PACDB	1208.8±229.5	1.43±0.44	0.70	0.23±0.00	4.35
9PACDB	1248.2±74.4	1.92±1.43	0.52	0.21±0.02	4.75
10PACDB	998.2±88.9	1.03±0.03	0.97	0.27±0.02	3.77
11PACDB	1113.7±45.5	1.75±0.10	0.57	0.22±0.01	4.52
12PACDB	1346.2±101.3	1.34±0.18	0.75	0.23±0.01	4.35
13PACDB	1420.8±99.2	1.44±0.06	0.69	0.16±0.01	6.36
14PACDB	1207.3±252.2	1.66±0.43	0.60	0.08±0.02	12.36
15PACDB	838.9±45.5	1.12±0.14	0.89	0.28±0.01	3.59
16PACDB	1005.6±272.9	2.86±1.48	0.35	0.22±0.00	4.56
17PACDB	1878.4±345.3	1.50±0.05	0.67	0.23±0.00	4.40
18PACDB	1232.2±84.8	2.03±0.68	0.49	0.26±0.02	3.78
19PACDB	713.2±70.3	3.43±1.58	0.29	0.26±0.02	3.86
20PACDB	1182.5±213.0	2.14±0.77	0.47	0.80±0.15	1.25
21PACDB	682.5±18.6	1.90±0.23	0.53	0.19±0.02	5.30

Moreover, total phenolic content, free radical scavenging activity, and anti-amylase activity were found in all 21 combined vegetable and fruit juices. The results showed that the highest observed total phenolic content was in the 1PACDB combination of ivy gourd, carrot, Chinese cabbage, guava, and pineapple at a ratio of 6:1:1:1:1, respectively, followed by 17PACDB (1:2:5:1:1), and then 13PACDB (2:3:3:1:1). The highest observed free radical scavenging activity was found in guavas, followed by 3PACDB (5:2:1:1:1), and then 5PACDB (4:2:2:1:1), while the highest observed anti-amylase activity was found in 2PACDB (5:1:2:1:1), followed by 14PACDB (2:4:2:1:1), and then 4PACDB (4:1:3:1:1).

Interestingly, vegetables and fruits are rich sources of biologically active agents such as vitamins, phenols, flavonoids, and polyphenols [13] that can affect change on the total biological activity in the vegetable and fruit mixtures they are a part of [30]. Therefore, the expected values for each combinatorial vegetable and fruit was used to compare with the observed values, to identify the effects from each combination, done according to the method used by Wang et al. (2011) [30]. For a rough approximation, each expected value was calculated by taking the sum of each individual ingredient for total phenolic content, free radical scavenging activity, and anti-amylase activity, shown in Table 1. If the observed values of each combination significantly exceeded its expected value, that particular formulation was identified as having a synergistic effect.

If the observed values were significantly less than the expected values, that juice mixture was identified as having an antagonistic effect among its ingredients. Moreover, if the expected value is equal to its observed value, it is indicated as an additive effect [37]. The observed and expected values of the 21 vegetable and fruit combinations from all assays are shown in Tables 3-5. The synergistic, antagonistic, and additive effects were found in the all combinations.

Table 3. Total phenolic content and effect types of vegetable and fruit combination.

Formula	Total phenolic contents (µg gallic acid equivalents/g sample)			
	E	O	Types of Interaction	*p*-value
1PACDB	1139.0±53.1	2217.5±146.8	Sy	0.01*
2PACDB	1087.9±68.9	1144.4±55.8	Ad	0.463
3PACDB	1092.8±53.1	916.4±175.7	Ad	0.307
4PACDB	1036.7±84.6	1239.5±186.1	Ad	0.296
5PACDB	1041.7±68.9	1277.5±285.3	Ad	0.374
6PACDB	1046.6±53.1	1014.3±24.8	Ad	0.517
7PACDB	985.5±100.3	1125.4±62.0	Ad	0.235
8PACDB	990.5±84.6	1208.8±229.5	Ad	0.334
9PACDB	995.5±68.9	1248.2±74.4	Ad	0.072
10PACDB	1000.4±53.1	998.2±88.9	Ad	0.979
11PACDB	934.4±116.0	1113.7±45.5	Ad	0.179
12PACDB	939.3±100.3	1346.2±101.3	Ad	0.056
13PACDB	944.3±84.6	1420.8±99.2	Sy	0.035*
14PACDB	949.3±68.9	1207.3±252.2	Ad	0.298
15PACDB	954.2±53.1	838.9±45.5	Ad	0.145
16PACDB	883.2±131.7	1005.6±272.9	Ad	0.625
17PACDB	888.2±116.0	1878.4±345.3	Ad	0.061
18PACDB	893.1±100.3	1232.2±84.8	Ad	0.068
19PACDB	898.1±84.6	713.2±70.3	Ad	0.141
20PACDB	903.1±68.9	1182.5±213.0	Ad	0.220
21PACDB	908.0±53.1	682.5±18.6	An	0.030*

*Indicates a significant difference between the observed value and expected value of each sample at *p*-value < 0.05, O is observed value, E is expected value, Sy is synergistic effect, Ad is additive effect, and An is antagonistic effect.

Table 4. Free radical scavenging and effect types of vegetable and fruit combination.

Formula	EC_{50} (%)			
	E	O	Interactions	P-value
1PACDB	1.39±0.34	1.37±0.43	Ad	0.952
2PACDB	1.56±0.41	1.49±0.17	Ad	0.855
3PACDB	1.54±0.49	0.41±0.03	Ad	0.083
4PACDB	1.72±0.48	1.80±0.67	Ad	0.903
5PACDB	1.70±0.56	0.75±0.14	Ad	0.146
6PACDB	1.69±0.65	3.41±0.25	Ad	0.072
7PACDB	1.88±0.55	0.96±0.01	Ad	0.144
8PACDB	1.87±0.63	1.43±0.44	Ad	0.51
9PACDB	1.85±0.72	1.92±1.43	Ad	0.959
10PACDB	1.84±0.80	1.03±0.03	Ad	0.287
11PACDB	2.04±0.62	1.75±0.10	Ad	0.581
12PACDB	2.03±0.71	1.34±0.18	Ad	0.313
13PACDB	2.01±0.79	1.44±0.06	Ad	0.412
14PACDB	2.00±0.87	1.66±0.43	Ad	0.671
15PACDB	1.99±0.95	1.12±0.14	Ad	0.328
16PACDB	2.20±0.70	2.86±1.48	Ad	0.63
17PACDB	2.19±0.78	1.50±0.05	Ad	0.336
18PACDB	2.18±0.86	2.03±0.68	Ad	0.871
19PACDB	2.16±0.94	3.43±1.58	Ad	0.432
20PACDB	2.15±1.02	2.14±0.77	Ad	0.992
21PACDB	2.14±1.10	1.90±0.23	Ad	0.79

*Indicates a significant difference between the observed value and expected value of each sample at *p*-value < 0.05, O is observed value, E is expected value, Sy is synergistic effect, Ad is additive effect; and An is antagonistic effect.

Table 5. Amylase inhibition and effect types of vegetable and fruit combination.

Formula	EC_{50} (%)			
	E	O	Interactions	P-value
1PACDB	0.30±0.00	0.20±0.02	Sy	0
2PACDB	0.39±0.01	0.07±0.01	Sy	0
3PACDB	0.30±0.00	0.23±0.00	Sy	0.001
4PACDB	0.49±0.01	0.09±0.04	Sy	0.001
5PACDB	0.40±0.00	0.21±0.03	Sy	0
6PACDB	0.31±0.01	0.26±0.00	Ad	0.229
7PACDB	0.58±0.02	0.31±1.00	Ad	0.731
8PACDB	0.49±0.01	0.23±0.00	Sy	0.001
9PACDB	0.40±0.00	0.21±0.02	Sy	0
10PACDB	0.31±0.01	0.27±0.02	Ad	0.138
11PACDB	0.67±0.03	0.22±0.01	Sy	0.002
12PACDB	0.58±0.02	0.23±0.01	Sy	0.002
13PACDB	0.49±0.01	0.16±0.01	Sy	0.001
14PACDB	0.40±0.00	0.08±0.02	Sy	0
15PACDB	0.32±0.01	0.28±0.01	Sy	0.102
16PACDB	0.77±0.04	0.22±0.00	Sy	0.002
17PACDB	0.68±0.03	0.23±0.00	Sy	0.002
18PACDB	0.59±0.02	0.26±0.02	Sy	0.002
19PACDB	0.50±0.01	0.26±0.02	Sy	0.001
20PACDB	0.41±0.01	0.80±0.15	Ad	0.11
21PACDB	0.32±0.01	0.19±0.02	Sy	0.003

*Indicates a significant difference between the observed value and expected value of each sample at p-value < 0.05, O is observed value, E is expected value, Sy is synergistic effect, Ad is additive effect; and An is antagonistic effect.

Table 6. The percentage of each effect from individual assay of all vegetable and fruit combinations.

	synergistic effects	antagonistic effects	additive effects
Total phenolic content	9.5	4.8	85.7
Free radical scavenging	0	0	100
Amylase inhibition	81.0	0	19.0

The results showed synergistic interactions of the total phenolic content assay in only 1PACDB and 13PACDB samples, while synergistic interactions were found for anti-amylase activity in 1PACDB, 2PACDB, 3PACDB, 4PACDB, 5PACDB, 8PACDB, 9PACDB, 11PACDB, 12PACDB, 13PACDB, 14PACDB, 15PACDB, 16PACDB, 17PACDB, 18PACDB, 19PACDB, and 21PACDB.

However, synergistic interactions of all combinations were not found by the free radical scavenging assay. The results showed the proportions of the combinatorial vegetables and fruits with synergistic interactions for 81%, 9.5%, and 0% for the anti-amylase assay, total phenolic content assay, and free radical scavenging assay, respectively (Table 6). This implies that consumption of most juice combinations will help to increase amylase inhibition. Moreover, most of them displayed additive interactions for free radical scavenging (100%), total phenolic content (85.7%), and amylase inhibition (19.0%), while antagonistic effects were only found for the 21PACDB mixture for total phenolic content (4.8%).

For free radical scavenging activity, additive interactions were found, by ABTS assay, for 100% of the combinations. Similar to the findings of a previous study, antioxidant capacities of individual and combined phenolic compounds were found in individuals and combinations of two or three phenolic compounds in fruits and vegetables, but synergistic interactions between them are not observed using ABTS assay [38]. However, it has been reported that antioxidant interactions from FRAP, DPPH, and ORAC assays of individual foods and their combinations showed the highest proportion for additive interactions, followed by antagonistic, and then synergistic interactions [30]. Moreover, antioxidant capacities and synergistic interactions of four phenolic compound combinations (gallic acid, protocatechuic acid, chlorogenic acid, and vanillic acid) from mango pulp were observed by DPPH assay, but antagonistic interactions were also found in some combinations, namely gallic acid with vanillic acid, as well as the combination of protocatechuic acid chlorogenic acid, and vanillic acid [39].

However, it has been reported that several non-phenolic compounds (i.e. tyrosine, tryptophan, cysteine, guanine, arachidonic acid, trioses glyceraldehyde, dihydroxyacetone, Fe^{+2}, Mn^{+2}, I^{-}, SO_3^{-2}, butanedione, alpha ionone, ascorbic acid, folic acid, folinic acid, NADH, pyridoxine, retinoic acid, thiamine, and trolox) can react with Folin-Ciocalteu reagent [40]. Similar to the ABTS assay, this Folin-Ciocalteu method is more appropriate for an assay measuring total antioxidant activity rather than measuring phenolic content, because most phenolic compounds have antioxidant activity [40-41].

Moreover, over 80% of all combinations of anti-amylase assays showed synergistic interactions in the present study. However, the synergistic anti-amylase response in food combinations may occur from interactions between phenolic agents and other phytochemicals. Some phenols may interact with amylase inhibitors in the juice combinations that lead to higher anti-diabetic activity via anti-amylase inhibition. Several studies have reported on synergistic interactions between plant derived phenolic agents and anti-diabetic drugs.

For example, combinations of gallic acid with anti-diabetic drugs or acarbose can have a synergistic interaction that leads to an increase in the inhibition of α-amylase [42]. Combinations of polyphenols and acarbose have been shown to have synergistic interactions that provide higher amylase inhibition [43]. Nowadays, information on the interaction between phenols and amylase inhibitors from natural foods is still unknown. However, α-amylase inhibition varies widely, not only with the number of phenols (i.e., flavonol, flavone, flavanone, flavanonol, and tannins), but most especially with bioactive plant compounds (i.e. water-soluble vitamins, water-insoluble fibers, and water-soluble dietary fibers) [44-45].

Further, PCA analysis of amylase inhibition, total phenolic content, and free radical scavenging activity of different individual plants and their combinations was performed, data are listed in Figure 1. The PCA results showed that the first principal component (PC1) and second principal component (PC2) explain 44.95% and 33.39% of the total variation, respectively. The PCA results show that the highest observed total phenolic content of the various combinations was 1PACDB, followed by 17PACDB, and then 13PACDB. The highest observed free radical scavenging activity was found in guavas, followed by 3PACDB, and then 5PACDB. The highest observed anti-amylase activity was in 2PACDB, followed by 14PACDB, and then 4PACDB. Interestingly, a very strong positive correlation was observed between the free radical scavenging activity and the amount of guava ($r = 0.883$, p-value = 0.000).

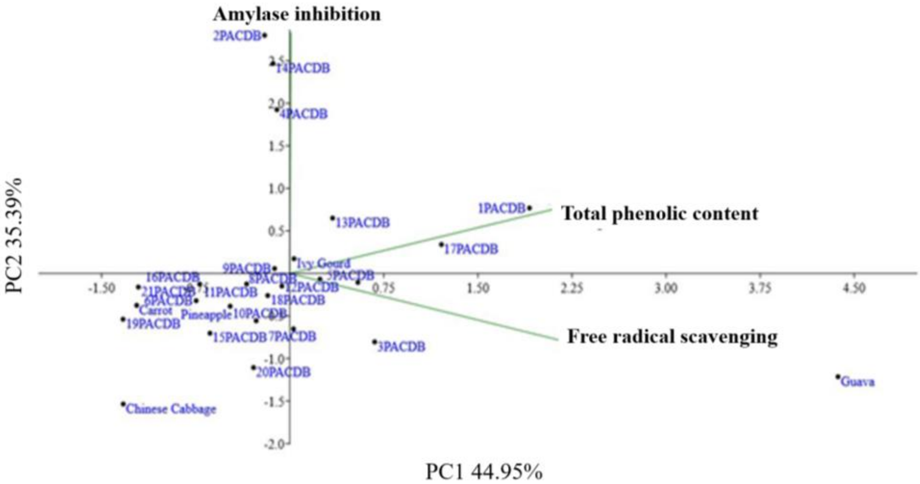

Fig. 1. Principal analysis component of the observed total phenolic contents, free radical scavenging and anti-amylase activity for guava, pineapple, ivy gourd, carrot, chinese cabbage juices, and the 21 vegetable and fruit mixtures.

Additionally, a weak negative correlation was observed between the total phenolic content and the amount of carrot (r = -0.389, P-value =0.049), while a moderate positive correlation was observed between the total phenolic content and the amount of guava (r = 0.424, *p*-value = 0.031).

It has been reported that plants are natural sources of vitamins, minerals, phytochemicals and fiber, known as being important components of healthy foods [13]. Guava fruit is an abundant source of several phytochemicals, such as vitamin A, vitamin C, iron, phosphorus, calcium, flavonoids, terpenes, and antioxidants [46]. Carrots have high levels of phenols and β-carotene, which help to promote good health and prevent chronic diseases [47-48]. β-carotene is a phytochemical agent in the carotenoid group, known as being a strong natural antioxidant [49-50]. Aqueous leaf and stem extracts of ivy gourd can help to control postprandial plasma glucose level by inhibition of α-amylase and α-glucosidase [51]. It contains alkaloids, reducing sugar, and saponins that display pharmacological properties [52].

Moreover, its leaf extract possesses strong antioxidant activity, reducing power ability, free radical scavenging activity, metal chelating ability, and β-carotene bleaching inhibition [53]. Pineapple contains a high amount of vitamin C, phenolic compounds, and carotenoids [54, 55]. The ethyl acetate and methanolic extracts of pineapple fruit show high levels of bioactive compounds (namely sinapic acid, daucosterol, 2-methylpropanoate, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, methyl 2-methylbutanoate, and triterpenoid ergosterol), and minerals (namely magnesium, potassium, and calcium), which indicate that this fruit has strong anti-diabetic properties [56]. Chinese cabbage contains carotenoids, glucosinolates, phenolic compounds, antioxidants, and it shows strong antioxidant activity, and anticancer activity [57].

However, antagonistic interactions may have a negative effect on health for those who consume these juice combinations. Several studies have focused on the synergistic interactions of food because it helps to increase quality and shelf life, as well as helps to reduce cost due to reducing the need to use artificial antioxidants [37]. Moreover, it has been reported that a mixture of pure phytochemicals, in vitro, can have synergistic interactions, increasing antioxidant capacity, anti-inflammation, anti-cancer activity, but in humans this will depend on bioaccessibility and bioavailability [58]. Therefore, our finding may help to design ingredient formulations for mixed fruit and vegetable juice, aiming to maximize antioxidant capacity, anti-amylase activity by optimizing the occurrence of synergistic and antagonist interactions.

4. Conclusion

Our study indicated that juice from ivy gourd, carrot, Chinese cabbage, guava, pineapple, and their 21 mixtures showed high total phenolic content, antioxidant capacity, and anti-amylase activity. Moreover, the three types of interactions (synergistic, additive, and antagonistic) were observed. Interestingly, the highest proportion of synergistic interactions was found for anti-amylase activity, followed by total phenolic content, while there is only one combinatorial juice that was found to have an antagonistic interaction, which was for total phenolic content. These data are useful for developing healthy food with high antioxidant and anti-amylase capacity.

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