

Effects of Simulated Gastrointestinal Digestion on Antioxidant Activities of Individual and Mixed Fruits

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Fruits are important part of dietary pattern and correlated with a lower risk of chronic diseases because they contain many natural antioxidants. The gastrointestinal digestion could affect on antioxidant activities of fruits. In this study, we investigated the effects of simulated gastrointestinal digestion on the antioxidant activities of individual and mixed fruits. In the gastric digestion, the FRAP values of all 11 fruits exhibited a decreased tendency, and the TEAC values showed an increased tendency. The TPC exhibited different results in the 11 fresh fruit samples. In fruits combination groups, no notable difference was found on the interaction with the FRAP values, and different interactions were detected with the TEAC values ($p < 0.05$). The gastric process did not make any difference on the TPC between the fruit combinations, but after the duodenal digestion the TPC of group 4 were notably decreased, and the TPC of groups 5 and 8 were increased ($p < 0.05$). Therefore, the different fruit extracts have different behaviors in tests of FRAP, TEAC, and TPC after the simulated digestion process. Further researches should be done to help explore the mechanisms of the different interactions.

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Key Words: fruit, antioxidant, interaction, simulated gastrointestinal digestion

INTRODUCTION

Fruits are important part of dietary pattern. Epidemiologic researches have reported that the increase intake of fruits is correlated with a lower risk of chronic diseases.¹⁻⁴ Antioxidants contained in fruits, such as polyphenols and vitamins, account for these health bioactivities.^{5,6} Polyphenols are pivotal contributors to the antioxidant capacities of fruits, and much evidence has been observed on the antioxidant potency and the prevention of some diseases.⁷⁻⁹ As critical plant food, fruits are very important sources of polyphenols and are commonly consumed. Polyphenols are ingested in the form of mixtures in fruit matrix. The complexity of the matrix affect the compounds release as well as their chemical physical properties, thus studying them individually is inefficient to evaluate the health effects of fruits, and to understand the possible interactions amongst the polyphenols within a food matrix.¹⁰ Interactions between antioxidative fruit components are significant, and the bioactivity in vivo could rely on many

factors, counting in food processing, metabolism, and in vitro activities in human. However, most studies are still confined to investigations on purified antioxidant mixtures of in vitro models. At present, there are few studies to investigate interactions between antioxidants in fruit matrix, especially those used in simulated digestion models. Previously, we have found 11 fruits to be the highest antioxidant activity.¹¹ Because the gastrointestinal digestion could affect on antioxidant activities of fruits, in the present study, we use a simulated gastrointestinal digestion model to study the influence of digestion on the antioxidant activity and phenolic content in food matrix, as well as to study the interactions between the antioxidants of fruit mixtures.

METHODS

Chemicals and Samples

The compounds 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ), 2,20-azinobis(3-ethylbenothiazoline-6-sulphonic acid) diammonium salt (ABTS), Folin-Ciocalteu's phenol reagent, pepsin (≥ 2400 U/mg), pancreatin ($4 \times$ USP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The

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bile salts were purchased from Fluka (St. Louis, MO, USA). The standard phenolic compounds were bought from Siyi Biotechnology Company (Chengdu, China). Acetic acid, methanol, potassium persulfate, hydrochloric acid, iron(II) sulfate heptahydrate, iron(III) chloride hexahydrate, sodium acetate and sodium carbonate were of analytical grade and purchased from Tianjin Chemical Factory (Tianjin, China). Deionized water was used throughout the experiment. Fruit samples were collected from supermarkets in Guangzhou, China.

Sample Preparation

The fresh fruits were cleaned with deionized water and then separated into analytical part (**Table 1**). Immediately, the separated fruit fractions were ground into fine particles with a

special grinder. Antioxidant components of fruit fractions were extracted in ultrasound. The ultrasound-assisted extraction was operated in an ultrasonic device (KQ-600E, electric power of 600 W, 40 kHz, Changzhou Nuoji Instrument Company, Changzhou, China) with a heating power of 800 W, equipped with a temperature control meter and a digital time counter. Briefly, 10 g precisely weighed sample was sonicated in the ethanol - water (60 mL, 50: 50, v/v) in a water bath (100 rpm, 37 °C) and shaking for 30 min. Then the mixture was centrifuged for 30 min at 4200 g, and the supernatant was recovered. Two milliliters of extracts were stored at -20 °C before using and detected within 24 h. The reaction process was conducted in a 100 mL round-bottom flask, which was fixed in a plastic rack placing in the ultrasonic device.

Table 1. Vegetable names and parts analyzed.

| English name | Scientific name | Analyzed part |
|----------------|--|-------------------|
| Red apple | <i>Malus pumila</i> Mill | Peel & pulp |
| Cherry | <i>Prunus avium</i> | Peel & pulp |
| Red grape | <i>Vitis vinifera</i> | Peel & pulp |
| Guava | <i>Psidium guajava</i> | Peel & pulp |
| Hawthorn | <i>Crataegus pinnatifida</i> Bge. Var. <i>major</i> N. | Peel & pulp |
| Kiwi fruit | <i>Actinidia chinensis</i> | Peel & pulp |
| Mango | <i>Mangifera indica</i> Linn | Pulp |
| Pomelo (green) | <i>Citrus maxima</i> | Pulp |
| Starfruit | <i>Averrhoa carambola</i> L. | Peel & pulp |
| Strawberry | <i>Fragaria ananassa</i> | Peel, pulp & seed |
| Sweetsop | <i>Annona squamosa</i> L. | Pulp |

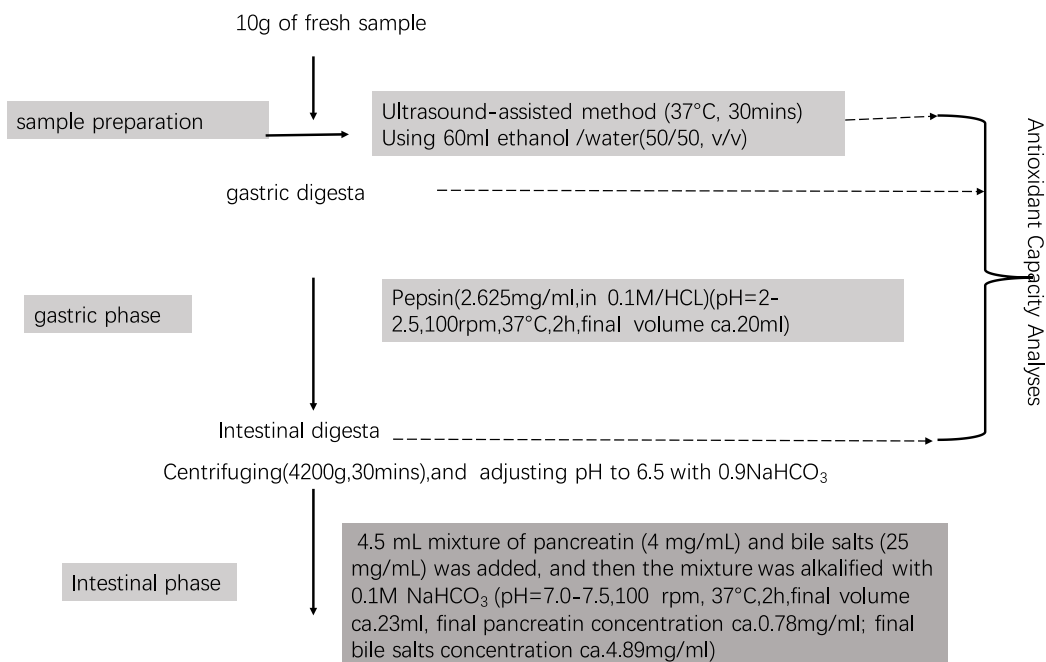


Figure 1. In vitro digestion procedure.

The Simulated Digestion Procedure

The simulated digestion model was adapted from Liang's study¹² and Tavares' study¹³ (Figure 1). Fruit samples (19 mL) were transferred to 50 mL plastic centrifuge tubes and mixed with a porcine pepsin preparation (1 mL). The samples were acidified to pH 2.0 then incubated at 37 °C in a water bath and shaken at 100 rpm for 2 h keeping in dark place. After gastric digestion, 2 mL of each sample was separated and stored at -20 °C. The pH was then adjusted to 6.5 with 0.9 M sodium bicarbonate. Then the samples were added in 4.5 mL mixture of pancreatin (4 mg/mL) and bile salts (25 mg/mL). The pH of each sample was alkalified to 7.4 with 1 M NaOH. Samples were incubated in a water bath (37 °C) and shaken at 95 rpm for 2 h keeping in dark place to perform the intestinal phase of the simulated gastrointestinal digestion process. After the intestinal digestion, 2 mL of each sample was extracted and stored at -20 °C, and the samples were analyzed within 24 h.

Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out according to the method described in the literature.¹⁴ In detail, the FRAP reagent was prepared from 20 mmol/L iron(III) chloride solution, sodium acetate buffer (300 mmol/L, pH 3.6) and 10 mmol/L TPTZ solution in a volume ratio of 10:1:1, respectively. The FRAP reagent was prepared freshly daily and kept in a water bath at 37 °C. One hundred microliters of the diluted sample was added to 3 mL of the FRAP reagent. The absorbance of the mixture was measured after 4 min at 593 nm. The standard curve was constructed using FeSO₄ solution, and the results were expressed as $\mu\text{mol Fe(II)/g}$ fresh weight (FW) of fruits.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay

The TEAC assay was carried out according to the method established in the literature.¹⁵ Briefly, the ABTS•+ stock solution was prepared from 2.45 mmol/L potassium persulfate and 7 mmol/L ABTS in a volume ratio of 1:1, and then incubated darkly at room temperature for 16 h and used within 2 days. The ABTS•+ operating solution was made up by diluting the stock solution with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. All samples were diluted to provide 20-80 percents inhibition of the blank absorbance. One hundred microliters of the diluted sample were mixed with 3.8 mL

ABTS•+ working solution, then the absorbance of the mixture was measured at 734 nm after incubation for 6 min. The percent of inhibition of absorbance was calculated at 734 nm. Trolox was used as a reference standard, and the results were expressed as $\mu\text{mol Trolox/g}$ fresh weight (FW) of fruits.

Determination of total phenolic content

Total phenolic contents were measured according to the literature.¹⁶ In detail, five hundred microliters of the diluted sample was added into 1% Folin-Ciocalteu reagent (2.5 mL). After 4 min, two milliliters of saturated sodium carbonate solution (75 g/L) was added. The absorbance of the mixture was detected at 760 nm after incubation for 2 h at room temperature. Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalents (mg GAE)/g fresh weight (FW) of fruits.

Statistical analysis

All the experiments were performed in triplicate, and the results were expressed as mean \pm SD (standard deviation). Statistical analysis was conducted using SPSS 16.0 and Excel 2003. Comparison of mean in multiple groups was conducted with homogeneity of variances and analysis of variance (ANOVA) by the software. Dunnett's multiple comparison test is used to analyze the difference before and after gastric digestion, and the difference of fresh samples and samples after duodenal phase. The difference was considered significant at $p < 0.05$.

RESULTS

Effects of the Simulated Digestion on the Antioxidant Capacities of Individual Fruits

Effects of the simulated digestion on the FRAP values of fruits

The initial FRAP values of the original fruit extracts varied considerably (2.61 - 51.73 $\mu\text{mol Fe(II)/g}$ FW) (Table 2). Within categories, hawthorn displayed the highest FRAP value ($51.73 \pm 1.27 \mu\text{mol Fe(II)/g}$ FW). Apple (red) showed the lowest total FRAP value ($2.61 \pm 0.77 \mu\text{mol Fe(II)/g}$) among these fruits.

Table 2. FRAP values ($\mu\text{mol Fe(II)/g}$ FW) for tested fruits before, and after the gastric and duodenal phases of the simulated digestion.

| Fruits | FRAP prior | FRAP gastric | FRAP duodenal |
|----------------|------------------|--------------------|--------------------|
| Apple (red) | 2.61 ± 0.77 | 1.79 ± 0.28 | 1.57 ± 0.09 |
| Cherry | 5.91 ± 0.40 | 5.92 ± 0.46 | 5.45 ± 0.79 |
| Grape (red) | 6.81 ± 0.09 | $6.18 \pm 0.16^*$ | $4.72 \pm 0.43^*$ |
| Guava | 36.25 ± 2.21 | $28.58 \pm 3.11^*$ | $28.85 \pm 2.73^*$ |
| Hawthorn | 51.73 ± 1.27 | $40.93 \pm 2.44^*$ | $42.31 \pm 2.63^*$ |
| Kiwi fruit | 11.64 ± 0.61 | 10.14 ± 0.82 | $9.62 \pm 0.22^*$ |
| Mango | 3.85 ± 0.51 | 3.13 ± 0.63 | 2.68 ± 0.76 |
| Pomelo (green) | 6.55 ± 1.73 | 4.64 ± 0.13 | 4.64 ± 0.79 |
| Star fruit | 15.84 ± 1.35 | 15.85 ± 0.77 | 14.61 ± 0.28 |
| Strawberry | 15.07 ± 0.09 | $13.64 \pm 0.39^*$ | $12.42 \pm 0.66^*$ |
| Sweetsop | 25.53 ± 0.88 | $19.15 \pm 0.75^*$ | $18.80 \pm 1.68^*$ |

* $p < 0.05$, it denotes significant difference from the ferric reducing antioxidant power (FRAP) value prior to the simulated digestion (n = 3).

Following the gastric digestion, the FRAP values of all 11 fruits exhibited a decrease tendency, except for cherry. In detail, FRAP values of the grape (red), guava, hawthorn, kiwi fruit, cherry and sweetsop were notably decreased ($p < 0.05$). After duodenal digestion, the FRAP values were minished further, among which the FRAP values of grape (red), guava, hawthorn, kiwi fruit, cherry and sweetsop showed a significant difference, comparing to the original extract ($p < 0.05$).

Effects of the simulated digestion on the TEAC values of fruits

Table 3 showed the antioxidant capacities prior to digestion. The TEAC values of the 11 original fruit extracts varied from 0.47 to 36.91 $\mu\text{mol Trolox/g FW}$ with the difference of 78.5-fold. In detail, hawthorn showed the highest TEAC value, about $36.91 \pm 3.80 \mu\text{mol Trolox/g FW}$, and apple (red) displayed the lowest TEAC values, about $0.47 \pm 0.07 \mu\text{mol Trolox/g FW}$. The antioxidant capacities of fruits were diversely affected in the simulated gastrointestinal digestion. In general, the TEAC values showed an increase tendency under gastrointestinal digestion, among which the changes of cherry, grapefruit, starfruit, strawberry, and sweetsop were significant different ($p < 0.05$). The TEAC values of guava, mango, and kiwi fruit increased slightly after gastric phase, and this change became notable after intestinal phase ($p < 0.05$).

Effects of the simulated digestion on the total phenolic content of fruits

The total phenolic content (TPC) exhibited different results in the 11 fresh fruit samples (0.15 - 11.85 mg GAE/g FW). In detail, hawthorn possessed the highest TPC, about 11.85 $\mu\text{mol GAE/g FW}$, and red apple displayed the lowest TPC, about 0.15 $\mu\text{mol GAE/g FW}$ (**Table 4**). Generally, the total phenolic content of the 11 fruits varied remarkably under gastrointestinal digestion.

The TPC values of red apple, mango, and grapefruit decreased after simulated gastric digestion, while notably increased after simulated duodenal digestion ($p < 0.05$). The TPC values of cherry and strawberry markedly decreased after gastric phase ($p < 0.05$), but after duodenal phase the TPC values recovered to predigestion levels.

There was not variation observed for the TPC values of red grape, guava, starfruit, and kiwi fruit under gastrointestinal digestion ($p > 0.05$). The most notable of variation was observed for hawthorn, which was 11.85 mg GAE/g FW in fresh sample, decreased from 3.84 mg GAE/g FW ($p < 0.05$) after the gastric phase to 3.32 mg GAE/g FW after the duodenal phase ($p < 0.05$). A similar trend was found in Bermúdez-Soto's study.¹⁷

Table 3. TEAC values ($\mu\text{mol Trolox/g FW}$) for tested fruits before, and after the gastric and duodenal phases of the simulated digestion.

| Fruits | TEAC prior | TEAC gastric | TEAC duodenal |
|----------------|------------------|--------------------|--------------------|
| Apple (red) | 0.47 ± 0.07 | 1.13 ± 0.20 | $3.24 \pm 0.31^*$ |
| Cherry | 2.07 ± 0.58 | $4.79 \pm 0.58^*$ | $6.99 \pm 0.30^*$ |
| Grape (red) | 3.98 ± 0.21 | $6.52 \pm 0.56^*$ | 3.10 ± 0.29 |
| Guava | 21.07 ± 0.93 | 25.30 ± 3.92 | $29.26 \pm 1.70^*$ |
| Hawthorn | 36.91 ± 3.80 | 28.57 ± 1.49 | 35.39 ± 2.00 |
| Kiwi fruit | 5.35 ± 0.54 | 6.21 ± 0.45 | $6.83 \pm 0.10^*$ |
| Mango | 3.02 ± 0.24 | 4.09 ± 0.66 | $5.37 \pm 0.14^*$ |
| Pomelo (green) | 2.53 ± 0.34 | $4.50 \pm 0.28^*$ | $6.56 \pm 0.14^*$ |
| Star fruit | 13.50 ± 0.42 | $18.29 \pm 0.53^*$ | $20.45 \pm 0.51^*$ |
| Strawberry | 8.60 ± 0.21 | $10.89 \pm 0.53^*$ | $12.13 \pm 0.49^*$ |
| Sweetsop | 19.45 ± 0.29 | $22.81 \pm 0.76^*$ | $25.48 \pm 1.17^*$ |

* $P < 0.05$, it denotes significant difference from the Trolox equivalent antioxidant capacity (TEAC) value prior to the simulated digestion ($n = 3$).

Table 4. Total phenolic content (mg GAE/g FW) for tested fruits before, and after the gastric and duodenal phases of the simulated digestion.

| Fruits | TPC prior | TPC gastric | TPC duodenal |
|----------------|------------------|-------------------|-------------------|
| Apple (red) | 0.15 ± 0.02 | 0.11 ± 0.03 | $0.20 \pm 0.02^*$ |
| Cherry | 0.61 ± 0.05 | $0.50 \pm 0.04^*$ | 0.64 ± 0.02 |
| Grape (red) | 0.49 ± 0.05 | 0.43 ± 0.02 | 0.53 ± 0.03 |
| Guava | 2.69 ± 0.12 | 2.36 ± 0.33 | 2.61 ± 0.23 |
| Hawthorn | 11.85 ± 0.05 | $3.84 \pm 0.96^*$ | $3.32 \pm 0.14^*$ |
| Kiwi fruit | 0.40 ± 0.04 | 0.44 ± 0.06 | $0.49 \pm 0.03^*$ |
| Mango | 0.19 ± 0.01 | 0.14 ± 0.03 | $0.30 \pm 0.02^*$ |
| Pomelo (green) | 0.59 ± 0.03 | 0.54 ± 0.01 | $0.69 \pm 0.05^*$ |
| Star fruit | 2.12 ± 0.14 | 1.99 ± 0.03 | 2.02 ± 0.06 |
| Strawberry | 1.18 ± 0.03 | $0.91 \pm 0.04^*$ | 1.17 ± 0.05 |
| Sweetsop | 3.10 ± 0.06 | $2.29 \pm 0.17^*$ | $2.53 \pm 0.25^*$ |

* $p < 0.05$, it denotes significant difference from the total phenolic content (TPC) value prior to the simulated digestion ($n = 3$).

Table 5. Eight fruit combinations.

| Sample | Fruit combinations |
|--------|---------------------------------|
| 1 | sweetsop + guava |
| 2 | sweetsop + starfruit |
| 3 | sweetsop + hawthorn |
| 4 | guava + starfruit |
| 5 | guava + hawthorn |
| 6 | starfruit + hawthorn |
| 7 | sweetsop + guava + starfruit |
| 8 | sweetsop + starfruit + hawthorn |

Effects of the Simulated Digestion on the Antioxidant Capacities of Fruits Combinations

To investigate the synergistic, additive, and antagonistic interactions of antioxidant activities among fruits, four kinds of fruits with stronger antioxidant activity, including sweetsop, guava, star fruit and hawthorn, were selected. Individual fruit extracts were mixed in groups as described in **Table 5**, and three antioxidant assays were used to evaluate their antioxidant capacities.

Effects of the simulated digestion on the FRAP values of fruits combinations

The observed FRAP value of the mixture was compared with

the expected value, which is the mathematical sum of the FRAP value obtained from the individual extracts. All combinations were based on the same total weight of the pair; for example, the FRAP value (observed) of a 1 g mixture of sweetsop and guava (0.5 g each when mixed at 1:1 v/v ratio) was compared with the mathematical sum of the FRAP value (expected) of 0.5 g of sweetsop and that of 0.5 g of guava. If the observed value was significantly lower than the expected value, was defined as an antagonistic interaction. While if the observed values were remarkably higher than the expected value obtained from of the same combinations of individual fruits ($p < 0.05$), a synergistic interaction might be occurred in the combinations.

Table 6. FRAP values ($\mu\text{mol Fe(II)/g FW}$) for tested fruits combinations before, and after the gastric and duodenal phases of the simulated digestion.^a

| Sample No. | FRAP prior | | FRAP gastric | | FRAP duodenal | |
|------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | Expected | Observed | Expected | Observed | Expected | Observed |
| 1 | 30.32 \pm 1.14 | 28.64 \pm 0.55 | 23.87 \pm 1.50 | 27.42 \pm 0.37 | 23.83 \pm 0.57 | 22.82 \pm 2.93 |
| 2 | 20.61 \pm 1.38 | 19.85 \pm 3.33 | 17.50 \pm 0.75 | 18.11 \pm 1.47 | 16.71 \pm 0.86 | 16.12 \pm 0.75 |
| 3 | 38.63 \pm 1.08 | 36.12 \pm 2.92 | 30.17 \pm 1.65 | 28.44 \pm 2.77 | 31.04 \pm 1.40 | 33.97 \pm 2.77 |
| 4 | 26.04 \pm 1.61 | 26.34 \pm 3.18 | 22.22 \pm 1.63 | 22.60 \pm 0.65 | 21.73 \pm 1.40 | 20.06 \pm 1.08 |
| 5 | 44.03 \pm 2.20 | 39.88 \pm 3.74 | 33.92 \pm 1.99 | 36.98 \pm 3.24 | 34.80 \pm 1.60 | 33.76 \pm 2.77 |
| 6 | 33.49 \pm 1.26 | 29.92 \pm 1.58 | 28.46 \pm 1.74 | 27.58 \pm 0.07 | 28.46 \pm 1.51 | 25.97 \pm 0.21 |
| 7 | 23.04 \pm 4.28 | 22.47 \pm 0.69 | 21.20 \pm 1.11 | 20.34 \pm 1.40 | 20.75 \pm 0.44 | 18.10 \pm 1.79 |
| 8 | 26.48 \pm 5.03 | 25.81 \pm 1.67 | 25.44 \pm 1.45 | 26.19 \pm 1.14 | 25.56 \pm 1.07 | 23.12 \pm 2.29 |

^a n = 3; Expected, expected value; Observed, observed value.

Table 7. TEAC values ($\mu\text{mol Trolox/g FW}$) for tested fruits combinations before, and after the gastric and duodenal phases of the simulated digestion.^a

| Sample No. | TEAC prior | | TEAC gastric | | TEAC duodenal | |
|------------|------------------|-------------------|------------------|-------------------|------------------|-------------------|
| | Expected | Observed | Expected | Observed | Expected | Observed |
| 1 | 20.26 \pm 0.50 | 18.78 \pm 0.63 | 24.05 \pm 2.26 | 24.29 \pm 0.42 | 27.37 \pm 0.72 | 26.17 \pm 0.18 |
| 2 | 16.47 \pm 0.28 | 16.44 \pm 2.74 | 20.55 \pm 0.44 | 21.80 \pm 0.85 | 22.96 \pm 0.50 | 21.95 \pm 3.62 |
| 3 | 28.18 \pm 1.84 | 22.47 \pm 1.24* | 25.62 \pm 1.25 | 28.81 \pm 1.69 | 30.37 \pm 1.81 | 36.54 \pm 1.07 |
| 4 | 17.28 \pm 0.67 | 18.19 \pm 0.79 | 21.79 \pm 2.13 | 20.88 \pm 0.15 | 24.85 \pm 1.11 | 20.25 \pm 0.86* |
| 5 | 28.99 \pm 2.30 | 24.37 \pm 1.55 | 25.95 \pm 2.11 | 30.88 \pm 1.00 | 32.78 \pm 0.55 | 33.27 \pm 2.58 |
| 6 | 25.21 \pm 2.07 | 22.18 \pm 0.03 | 23.30 \pm 0.59 | 29.62 \pm 0.63* | 28.06 \pm 0.88 | 33.82 \pm 2.04 |
| 7 | 18.01 \pm 0.47 | 17.29 \pm 0.47 | 22.13 \pm 1.60 | 21.24 \pm 1.53 | 25.06 \pm 0.62 | 21.90 \pm 1.81 |
| 8 | 23.28 \pm 1.35 | 23.12 \pm 2.60 | 23.08 \pm 0.73 | 27.83 \pm 1.36 | 27.16 \pm 1.13 | 29.62 \pm 1.84 |

^a n = 3, * comparison with expected value, $p < 0.05$.

Table 8. TPC (mg GAE/g FW) for tested fruits combinations before, and after the gastric and duodenal phases of the simulated digestion.^a

| Sample No. | TPC prior | | TPC gastric | | TPC duodenal | |
|------------|-------------|--------------|-------------|-------------|--------------|--------------|
| | Expected | Observed | Expected | Observed | Expected | Observed |
| 1 | 2.89 ± 0.09 | 2.39 ± 0.56 | 2.32 ± 0.20 | 2.76 ± 0.08 | 2.57 ± 0.13 | 2.51 ± 0.26 |
| 2 | 2.61 ± 0.09 | 2.50 ± 0.34 | 2.14 ± 0.07 | 2.40 ± 0.25 | 2.27 ± 0.10 | 2.25 ± 0.51 |
| 3 | 7.47 ± 0.05 | 4.12 ± 0.30* | 3.07 ± 0.47 | 3.93 ± 0.33 | 2.93 ± 0.11 | 4.34 ± 0.16 |
| 4 | 2.40 ± 0.13 | 2.23 ± 0.17 | 2.18 ± 0.17 | 2.19 ± 0.07 | 2.32 ± 0.14 | 1.94 ± 0.02* |
| 5 | 7.27 ± 0.07 | 4.18 ± 0.27* | 3.10 ± 0.63 | 4.11 ± 0.14 | 3.02 ± 0.17 | 4.01 ± 0.10* |
| 6 | 6.99 ± 0.07 | 3.83 ± 0.10* | 2.92 ± 0.49 | 3.75 ± 0.03 | 2.67 ± 0.12 | 3.83 ± 0.36 |
| 7 | 2.64 ± 0.10 | 2.47 ± 0.06 | 2.21 ± 0.14 | 2.30 ± 0.16 | 2.39 ± 0.08 | 2.22 ± 0.26 |
| 8 | 5.69 ± 0.06 | 3.00 ± 0.27* | 2.71 ± 0.32 | 2.97 ± 0.13 | 2.63 ± 0.04 | 3.42 ± 0.30* |

^a n = 3* comparison with expected value, $p < 0.05$

From **Table 6**, it could be found that there were only additive interactions observed among all combinations, no notable difference between the two values showed an additive interaction. ($p > 0.05$).

Effects of the simulated digestion on the TEAC values of fruits combinations

From **Table 7**, an antagonistic interaction was detected in the initial combination of sweetsop and hawthorn at prior digestion ($p < 0.05$). In gastric phase, the observed value was significantly higher than the expected value in the combination of starfruit and hawthorn ($p < 0.05$). In duodenal phase, the combination of guava and starfruit resulted in an antagonistic interaction ($p < 0.05$).

Effects of the simulated digestion on the total phenolic content of fruits combinations

The results indicated that the combination of fruits resulted in different changes of total phenolic content (**Table 8**). At prior digestion, almost all fruit combinations displayed decrease of the total phenolic content. In detail, statistical differences were detected in the combinations of groups 3, 5, 6 and 7. To our surprise, the gastric process did not make any difference on the phenolic contents between the fruit combinations. However, different interactions were found after the duodenal digestion. In detail, the TPC of group 4 were notably decreased, and the TPC of groups 5 and 8 were increased ($p < 0.05$).

DISCUSSION

Previously, several studies reported that antioxidant activities of fruits could be reduced after digestion,¹⁷⁻¹⁹ and this result was in agreement with our study. However, Ryan and his colleagues reported different findings.²⁰ In their study, the FRAP values of fruit juices, such as red grape juices and pomegranate juice, were notably increased after digestion ($p < 0.05$).

Certain non-antioxidant food compositions, such as amino acids, uronic acids, and sugars, could be released after simulated gastrointestinal digestion, and showed positive interference effects in TEAC, therefore leading to overestimated values.²¹ Interestingly, the TEAC value of red

grape was dramatically increased ($p < 0.05$), however after intestinal digestion the result were lower than predigestion level. In addition, the TEAC value of hawthorn decreased after gastric phase while recovered to predigestion levels, and the changes among the three phases were not significant ($p > 0.05$). This was also reported by Ryan and his colleague²⁰ who suggested the possibility that the compounds were resistant to changes in pH value and enzymatic hydrolysis. Furthermore, Bermúdez-Soto et al.¹⁷ indicated the possibility that structural transformation of phenolic compounds might not be detected by the same analytical method. When fruits were combined, different interactions were detected, which might imply that when two fruits are consumed simultaneously, the ultimate antioxidant capacity might not line with those of the individual fruits. That is to say that fruit interactions could play an important effect in the ultimate antioxidation of food combinations.

Ryan and Prescott²⁰ suggested that this might depend on a structural transformation in the phenolic compounds which make them undetectable by the individual HPLC phenolic compound analysis which Bermúdez-Soto et al.¹⁷ had used while did not measure the phenolic compounds. The results are in agreement with those of several studies which reported that polyphenols are highly sensitive to the mild alkaline conditions in the small intestine and that the change in their antioxidant activity results in their structure modifications.^{22,23} In Bermúdez-Soto's study,¹⁷ they found that some phenolic compounds were extremely unstable under alkaline conditions. As a result of the bases, the structures and bioactivities of those unstable compounds changed in the intestinal phase. This might explain the results detected in our study.

In our previous study, phenolics have been found to be major contributors to the total antioxidation of plant foods.^{9,11} The positive correlation between the antioxidant capacity and total phenolic content of fruits has been previously reported. Hence the synergistic antioxidant reaction in food combinations might not necessarily only generate from the polyphenols interacting with each other but possibly from interactions with other plant compounds.

CONCLUSIONS

This study indicated that different fruit extracts have different behaviors in tests of FRAP, TEAC, and TPC after the *in vitro* digestion process. Although the *in vitro* simulated gastrointestinal process method could not mimic the *in vivo* conditions, it might provide data on the stability under gastrointestinal digestion. Further researches involving the relationship between polyphenols, antioxidant activity, food matrix and digestion under physiological conditions, including cell models and *in vivo* studies, are warranted.

CONFLICT OF INTEREST

The authors claim no conflict of interest in this study.

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