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RESEARCH ARTICLE

The total antioxidant capacity, total phenolics content and starch hydrolase inhibitory activity of fruit juices following pepsin (gastric) and pancreatin (duodenal) digestion

Nilakshi Jayawardena[1] · Mindani I. Watawana[1] · Viduranga Y. Waisundara[1]

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Abstract The objective of the study was to evaluate the stability of the antioxidant activity, total phenolics content and starch hydrolase inhibitory activities of 22 commercially available fruit juices using an in vitro digestion model. These are important parameters as far as consumers are concerned due to their associated therapeutic properties. The Oxygen Radical Absorbance Capacity (ORAC) assay, Ferric Reducing Antioxidant Power (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays as well as the inhibition of α-amylase and α-glucosidase were carried out. All 22 fruit juices were a significant source of polyphenols with values varying between 235 and 389 μg gallic acid equivalents per mL. Twelve out of the 22 juices showed a statistically significant increase ($P < 0.05$) in polyphenol content following the duodenal phase of digestion. ORAC values correlated better with the total phenolics content ($R^2 = 0.975$) as compared with FRAP ($R^2 = 0.893$), DPPH ($R^2 = 0.821$) and ABTS ($R^2 = 0.752$). IC_{50} values of the α-amylase inhibitory activity kept increasing following both digestion phases, indicating the reduced ability of the juices to inhibit this enzyme. However, the IC_{50} values of the α-glucosidase inhibitory activity kept decreasing, indicating a better efficacy to inhibit this enzyme. Overall, all fruit juices were observed to be a significant source of antioxidants with starch hydrolase inhibitory properties.

Keywords α-Amylase · α-Glucosidase · ABTS · DPPH · FRAP · ORAC

1 Introduction

Free radicals play an important role in food and chemical material degradation and contribute also to many physiological disorders in humans. Highly reactive free radicals and oxygen species present in biological systems can oxidize nucleic acids, proteins and lipids, initiating degenerative diseases (Jalil and Ismail 2008; Ye and Song 2008). In this aspect, antioxidants are known to significantly delay or prevent the oxidation of easily oxidizable substrates. Out of all food products, plant-based food products are known to contain high concentrations of numerous redox-active antioxidants, such as polyphenols, carotenoids, tocopherols, glutathione, ascorbic acid and enzymes with antioxidant activity, which fight against hazardous oxidative damage of plant cell components (Schaffer et al. 2007). In comparison, in animal cells, antioxidant production is much more limited and oxidative damage is involved in the pathogenesis of most chronic degenerative diseases and aging (Rose et al. 1986). Thus, due to this association, epidemiological studies have shown that diets rich in vegetables and fruits have been able to significantly reduce the incidence of chronic diseases such as diabetes, cancer and cardiovascular disease. Increasing their consumption has been identified as a practical approach for chronic diseases prevention

✉ Viduranga Y. Waisundara
viduranga@gmail.com

[1] National Institute of Fundamental Studies, Hantane Road, Kandy 20000, Sri Lanka

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(Liu 2003; Riboli and Norat 2003). In addition to antioxidant properties, incorporation of starch hydrolase inhibitors into the diet has also been clinically identified as an effective remedy of preventing chronic diseases (Liu et al. 2011). These inhibitors retard glucose absorption through inhibition of the starch-hydrolyzing enzymes α-amylase and α-glucosidase, present in the small intestinal brush border.

Recent studies have begun to demonstrate that a number of commercially available fruit and vegetable juices display high total antioxidant capacity (TAC) when quantified using biochemical assays (Huang et al. 2005). In addition, a number of studies also present a measure of the total polyphenol content of food products in order to draw comparisons with other similar products, and to provide more detailed information about this sub-group of antioxidants comprising flavanoids, lignins and tannins (Huang et al. 2005; Bravo 1998). Polyphenolic compounds are thought to be particularly important in the pathologies of heart disease, hypertension and age-related degeneration (Zhao 2009; Zern and Fernandez 2005). Additionally, it is important to quantify the proportion of the ingested antioxidant capacity and starch hydrolase inhibitory activity which are available for use in the biological system. This is referred to as 'bioaccessibility' and refers specifically to the quantity of antioxidants or starch hydrolase inhibitors which are released from the food matrix and presented to the intestinal brush border for transport into the cell (Garrett et al. 2000). This differs from 'bioavailability' which refers to the quantity of antioxidants which actually pass through the cell membrane and are available for use within the cell (Granado-Lorencio et al. 2007).

Given the significant rate of urbanization throughout the world, consumers tend to purchase food products off the supermarket shelves (Attanapola et al. 2012; Illangasekera et al. 2004). From the perspective of fruits and vegetables, consumer trends have highlighted that in Sri Lanka, fruit juices are purchased more frequently than fresh fruits themselves (Illangasekera et al. 2006; Mulgirigama and Illangasekera 2000). Bearing this trend in mind as well as the rise in the incidence of chronic diseases, only a few studies have been carried out to date on the therapeutic capabilities of commercially available fruit juices. Thus, the aim of this research was to quantify the TAC and starch hydrolase inhibitory activity of 22 commercially available fruit juices and assess the stability of their TAC and starch hydrolase inhibitory activity after the gastric and duodenal phases of an in vitro digestion model using the Oxygen Radical Absorbance Capacity (ORAC) assay, Ferric Reducing Antioxidant Power (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS·+) radical scavenging assays as well as high-throughput measurements of the inhibition of α-amylase and α-glucosidase.

2 Materials and methods

Anhydrous sodium carbonate, Folin–Ciocalteu's phenol reagent, KH_2PO_4 and K_2HPO_4 were obtained from Merck (Darmstadt, Germany). 4,6-tripyridyl-s-triazine (TPTZ), gallic acid and trolox were purchased from Arcos Organics (Morris Plains, NJ, USA). All other chemicals were of analytical grade and obtained from Sigma Chemicals (St. Louis, MO, USA). The fruit juices were purchased from Keells Supermarket, Kandy, Sri Lanka and Cargills Food City, Kandy, Sri Lanka. The list of fruit juices and their sample codes are as follows: Onjus Orange juice (O1), Dimes Orange juice (O2), Berri Orange juice (O3), My Juicee Orange juice (O4), Robinsons Orange juice (O5), Pfanner Orange juice (O6), Fontana Mango juice (M1), Tropicana Mango juice (M2), My Juicee Mango juice (M3), Yummi Mango juice (M4), Berri red Apple juice (A1), Pfanner red Apple juice (A2), Dimes red Apple juice (A3), Fontana red Apple juice (A4), My Juicee red Apple juice (A5), Berri Grape juice (G1), Fontana Grape juice (G2), Fontana Pineapple juice (Pi1), Onjus Lime juice (Li1), Fontana Lemon juice (Le1), Yummi Passion fruit juice (Pa1), Yummi Woodapple juice (W1). The respective times to expiry of the fruit juices are shown in Table 1. Both value and premium brands were selected based on global consumer studies, which included fresh and concentrated varieties and are indicated in Table 1 as well. For Apple juices, only the red Apple juices were selected since the red Apple juices were more popular than the green Apple juices.

2.1 In vitro digestion procedure

The in vitro digestion model was adapted from Ryan et al. (2008). In brief, fruit juice samples were transferred to clean amber bottles and mixed with saline (balanced salt solution) to create a final volume of 20 mL. A blank sample consisting of phosphate buffered saline (PBS) at pH 7.0 was subjected to the digestion procedures in order to eliminate any interferences coming from the reagents. The samples were acidified to pH 2.0 with 1 mL of a porcine

Table 1 List of fruit juices and their time to expiry

Fruit	Sample code	Time to expiry (months)	Value (V)/premium (P) brand
Orange	O1	8	P
	O2	7	P
	O3	6	P
	O4	4	V
	O5	6	P
	O6	8	P
Mango	M1	1	P
	M2	4	P
	M3	3	V
	M4	2	V
Apple	A1	2	P
	A2	8	P
	A3	8	P
	A4	3	P
	A5	5	V
Grape	G1	2	P
	G2	4	V
Pineapple	Pi1	6	P
Lime	Li1	5	V
Lemon	Le1	1	P
Passion fruit	Pa1	4	V
Wood apple	W1	2	V

pepsin preparation (0.04 g pepsin in 1 mL 0.1 M HCl) and incubated at 37 °C in a shaking water bath at 95 rpm for 1 h. After gastric digestion, 500 μL of each sample was extracted and stored at −20 °C The pH was then increased to 5.3 with 0.9 M sodium bicarbonate followed by the addition of 200 μL of bile salts glycodeoxycholate (0.04 g in 1 mL saline), taurodeoxycholate (0.025 g in 1 mL saline), taurocholate (0.04 g in 1 mL saline) and 100 μL of pancreatin (0.04 g in 500 μL saline). The pH of each sample was increased to 7.4 with 1 M NaOH. Samples were incubated in a shaking water bath at 95 rpm at 37 °C for 2 h to complete the intestinal phase of the in vitro digestion process. After the intestinal phase, 500 μL of each sample was extracted and stored at −20 °C. All samples were analyzed within 2 weeks.

2.2 Total phenolics content

The total phenolics content was measured according to Wootton-Beard et al. (2011). In brief, the supernatant (0.2 mL) was added to 1.5 mL of Folin–Ciocalteu reagent (1:10 v/v with water). The mixture was allowed to equilibrate for 5 min and mixed with 1.5 mL of 60 g/L of sodium carbonate solution. The amount of sodium carbonate was determined based on the titratable acidity of the fruit juices measured using the method by Chen and Liu (2000). The titratable acidity of all the juices were 6.45–6.55 mL. The absorbance was read at 725 nm after incubation for 30 min, using the respective solvent as a blank. The results are expressed as μg gallic acid equivalents per mL of sample (μg GAE/mL).

2.3 Antioxidant activity assays

The ORAC assay was carried out according to the method by Prior et al. (2003) in 96-well microplate format using a Thermo Scientific Multiskan FC Microplate Reader. Results were expressed as μmol trolox equivalents per mL of sample (μmol TE/mL). The DPPH radical scavenging ability was evaluated using the method by Brand-Williams et al. (1995). The percentage inhibition was calculated against a control and compared to an ascorbic acid standard curve (0–1000 μM). The FRAP assay was conducted according to the method by Benzie and Strain (1996). The total antioxidant capacity of samples was determined against a standard of ferrous sulphate (1000 μM) whose FRAP value was known. The ABTS·+ free radical scavenging activity was evaluated using a methodology previously reported by Ozgen et al. (2006). The percentage inhibition was calculated against a control and compared to a Trolox standard curve (10–100 mM).

2.4 Assays of α-amylase and α-glucosidase inhibitory activities

The α-amylase inhibitory activity of the fruit juices was carried out according to the method by Liu et al. (2011), while the α-glucosidase inhibitory activity was carried out according to the method by Koh et al. (2009). Acarbose was used as the positive control, while PBS was used as the negative control for both assays, where it was subjected to the same in vitro digestion procedures as the fruit juices. This was carried out in order to eliminate any interference and/or influence from the addition of pancreatin and bile salts from the starch hydrolase inhibition reactions. The data for both enzyme inhibitory activities were expressed as IC_{50}, micrograms Acarbose Equivalents per milliliter of sample (μg AE/mL).

2.5 Statistical analysis

All data are presented as mean ± standard error mean (SEM) of at least three independent

experiments (n ≥ 3), where each experiment had a minimum of three replicates of each sample. The rationale for choosing SEM to depict the error was that it better represents the precision of the mean value and takes into account both standard deviation and sample size. For comparisons between samples, data were analyzed by ANOVA and Tukey's multiple comparison test (SPSS, version 17). A probability of 5 % or less was accepted as statistically significant.

3 Results

The total phenolics contents are shown in Table 2. All 22 fruit juices were a significant source of polyphenols; however the total amount varied significantly between the various types of juices (235–389 μg GAE/mL). Prior to digestion, the apple and grape juices had the highest amount of polyphenols. Following the gastric phase, there was a statistically significant increase ($P < 0.05$) in the total phenolics content in Dimes Orange juice and Berri Apple juice. A statistically significant decrease ($P < 0.05$) was observed in My Juicee Mango juice. A more obvious increase in the total phenolics content was observed following the duodenal phase of digestion where 12 out of the 22 juices showed a statistically significant increase ($P < 0.05$). The PBS blank did not indicate the presence of phenolic compounds, eliminating the interference coming from the reagents used for the digestion procedure. Thus, the source for the increase in phenolic compounds could be hypothesized as coming from the sediment/solid material of the fruit juices, where more phenolic compounds were released into the liquid supernatant.

Results of the ORAC, FRAP, DPPH and ABTS radical scavenging assays are shown in Tables 3 and 4. The PBS blank did not indicate any antioxidative activity across any of the assays, thus, eliminating the interference coming from the reagents used for the digestion procedure. The ORAC values correlated better with the total phenolics content ($R^2 = 0.975$) as compared with FRAP ($R^2 = 0.893$), DPPH ($R^2 = 0.821$) and ABTS ($R^2 = 0.752$). The ORAC values of the juices varied between 846 and 1848 μmol TE/mL. Within categories, the apple, mango and grape juices had the highest ORAC values. The ORAC values of six out of 22 juices increased statistically

Table 2 The total phenolics contents of fruit juices prior to digestion and following the gastric and duodenal phases

Sample code	Prior (μg GAE/mL)	Gastric (μg GAE/mL)	Duodenal (μg GAE/mL)
O1	259.6 ± 12.6	270.3 ± 13.6	301.5 ± 16.5*
O2	268.4 ± 14.5	284.6 ± 12.4*	325.6 ± 15.8*
O3	235.4 ± 13.8	265.9 ± 12.5	285.6 ± 14.9*
O4	248.9 ± 12.5	258.4 ± 15.3	274.3 ± 16.8*
O5	259.1 ± 10.7	274.6 ± 13.2	285.9 ± 15.2*
O6	236.8 ± 11.4	246.9 ± 15.4	265.8 ± 16.3
M1	369.4 ± 13.9	371.2 ± 13.6	421.3 ± 15.8*
M2	325.1 ± 12.4	326.9 ± 12.5	401.2 ± 12.7*
M3	339.5 ± 10.9	305.8 ± 11.9*	413.6 ± 14.8*
M4	247.6 ± 16.5	226.5 ± 12.4	195.6 ± 12.9*
A1	349.2 ± 349.2	368.5 ± 13.2	328.6 ± 15.4*
A2	326.9 ± 16.2	329.4 ± 12.5	356.4 ± 16.2*
A3	364.9 ± 12.4	379.6 ± 12.6	385.6 ± 15.4*
A4	384.9 ± 13.8	389.5 ± 13.5	389.6 ± 15.8*
A5	365.2 ± 15.6	384.2 ± 14.3	412.1 ± 13.4*
G1	388.6 ± 12.0	395.8 ± 13.6	385.3 ± 12.9
G2	382.1 ± 12.8	392.4 ± 12.6	402.1 ± 12.5
Pi1	241.3 ± 13.8	240.5 ± 13.9	285.6 ± 12.8*
Li1	255.3 ± 13.9	253.6 ± 12.8	267.5 ± 13.6*
Le1	239.8 ± 14.7	244.5 ± 12.5	286.5 ± 15.4*
Pa1	248.6 ± 13.9	251.3 ± 12.6	259.5 ± 15.6
W1	364.9 ± 15.8	364.9 ± 12.3	386.4 ± 13.6

Values represent mean ± standard error mean of 3 or more independent repetitions

* $P < 0.05$, denotes statistically significant difference as compared with prior to in vitro digestion (ANOVA, Tukey's test)

Table 3 The ORAC and FRAP activities of the fruit juices prior to digestion and following the gastric and duodenal phases

Sample code	ORAC			FRAP		
	Prior (μmol TE/mL)	Gastric (μmol TE/mL)	Duodenal (μmol TE/mL)	Prior (μmol TE/mL)	Gastric (μmol TE/mL)	Duodenal (μmol TE/mL)
O1	865.4 ± 20.5	985.4 ± 21.6*	1256.9 ± 33.7*	1357.6 ± 45.3	1652.3 ± 41.6*	1658.3 ± 36.8*
O2	985.7 ± 19.4	1024.3 ± 37.6	1436.2 ± 25.1*	1466.3 ± 37.3	1943.2 ± 42.7*	2069.8 ± 41.2*
O3	811.5 ± 18.3	965.7 ± 28.4*	1149.6 ± 26.4*	1065.4 ± 41.2	1653.9 ± 39.4*	2149.5 ± 40.9*
O4	846.2 ± 17.6	954.3 ± 21.9*	1240.3 ± 22.8*	1354.9 ± 44.6	1845.6 ± 28.7*	1859.3 ± 38.6*
O5	953.4 ± 21.6	1022.4 ± 30.4	1127.4 ± 24.1*	1287.2 ± 39.0	1739.2 ± 26.1*	1811.5 ± 37.5*
O6	924.8 ± 25.7	974.5 ± 32.1	1036.2 ± 34.5	1294.3 ± 43.5	1634.3 ± 24.8*	1714.3 ± 41.1*
M1	1437.5 ± 33.9	1465.4 ± 23.9	1769.2 ± 25.1*	1587.9 ± 42.2	1986.3 ± 33.9*	2046.9 ± 43.9*
M2	1284.6 ± 37.4	1259.6 ± 27.5	1349.6 ± 24.1	1699.2 ± 38.7	1893.6 ± 37.1*	2459.6 ± 38.0*
M3	1344.8 ± 28.6	1257.6 ± 18.2	1425.3 ± 37.6*	1756.3 ± 43.6	1978.6 ± 29.4*	2153.6 ± 46.9*
M4	1101.5 ± 18.7	965.4 ± 17.9	872.0 ± 21.5	1543.6 ± 24.5	2015.3 ± 33.4*	2217.4 ± 44.1*
A1	1369.5 ± 23.3	1456.8 ± 19.3*	1325.3 ± 30.6	1879.6 ± 49.5	2468.3 ± 34.5*	2459.3 ± 48.2*
A2	1387.4 ± 34.2	1395.7 ± 20.8	1405.3 ± 38.2*	1987.3 ± 48.2	2234.9 ± 29.7*	2369.4 ± 39.7*
A3	1498.6 ± 30.8	1518.9 ± 22.6	1622.4 ± 36.5*	1993.6 ± 47.6	2105.3 ± 34.9*	2418.3 ± 38.4*
A4	1559.4 ± 29.1	1562.4 ± 23.1	1684.3 ± 33.2*	1894.2 ± 47.3	1953.3 ± 35.1*	2357.1 ± 41.8*
A5	1454.6 ± 30.5	1574.3 ± 34.9	1649.2 ± 27.3	1936.2 ± 48.1	2159.4 ± 29.6*	2065.3 ± 42.5*
G1	1847.8 ± 38.2	1987.3 ± 35.1*	1842.3 ± 25.8	2593.4 ± 51.3	2596.1 ± 37.5	2649.3 ± 41.1
G2	1745.1 ± 39.4	1854.2 ± 24.7*	1965.2 ± 24.9*	2635.4 ± 54.3	2879.1 ± 41.6*	2947.1 ± 49.2*
Pi1	758.6 ± 22.5	754.3 ± 22.6	869.3 ± 23.5	1022.6 ± 25.9	1235.4 ± 22.4*	1694.8 ± 39.2*
Li1	948.3 ± 18.5	924.6 ± 28.1	1034.2 ± 31.7*	1134.2 ± 29.1	1246.3 ± 21.3*	1354.9 ± 24.8*
Le1	958.6 ± 19.6	968.2 ± 31.6	1123.4 ± 34.5*	1165.9 ± 25.3	1198.5 ± 20.9*	1294.6 ± 23.4*
Pa1	1054.6 ± 21.4	1125.0 ± 33.4	1254.3 ± 26.1*	1298.3 ± 24.6	1315.4 ± 19.7*	1457.3 ± 22.5*
W1	1657.2 ± 37.4	1643.9 ± 32.9	1743.0 ± 34.9	1593.4 ± 28.1	1694.2 ± 20.6*	1846.9 ± 21.8*

Values represent mean ± standard error mean of 3 or more independent repetitions

* $P < 0.05$, denotes statistically significant difference as compared with prior to in vitro digestion (ANOVA, Tukey's test)

significantly ($P < 0.05$) following the gastric digestion process. The ORAC values of additional eight juices increased statistically significantly ($P < 0.05$ following the duodenal phase resulting in a total of 14 juices with a statistically significant increase ($P < 0.05$) as compared to prior to digestion. Post digestion values are regarded as more important for these juices because the in vitro digestion model indicates the availability of fruit juice antioxidants in a biological system (Ryan et al. 2008). Overall, the greatest increase in the ORAC values was observed after the duodenal phase with only a small decrease after the gastric phase. None of the ORAC values had decreased during the digestion processes with the exception of Yummi Mango juice. The changes in the FRAP values showed a clearer trend than the ORAC values of the fruit juices between the two digestion phases, with the exception of Berri Grape juice. This could be due to the fact that this particular product was close to its expiry date. As shown in Table 3, the FRAP values showed a statistically significant increase ($P < 0.05$) from the gastric phase itself, which increased further during the duodenal phase. For both ORAC and FRAP values, the value brands had a higher antioxidant capacity than the premium brands. As for the ABTS and DPPH radical scavenging activities, there was no statistically significant difference ($P < 0.05$) between these values before and after digestion processes observed. The values remained consistently high throughout the entire study. Overall, the orange juices had the highest percentage of inhibition of the ABTS and DPPH radicals.

The α-amylase and α-glucosidase inhibitory activities of the fruit juices prior to digestion as well as afterwards are shown in Table 5. The IC_{50} values of the acarbose for α-amylase and α-glucosidase were 3.7 ± 0.9 and 2.1 ± 0.2 μg/mL, respectively. The IC_{50} values of the α-amylase inhibitory activity kept increasing following the gastric and duodenal digestion phases, indicating the reduced ability of the juices to inhibit the enzyme activity. However, the IC_{50} values of the α-glucosidase inhibitory activity of

Table 4 The DPPH and ABTS scavenging activities of the fruit juices prior to digestion and following the gastric and duodenal phases

Sample code	DPPH			ABTS		
	Prior (% inhibition)	Gastric (% inhibition)	Duodenal (% inhibition)	Prior (% inhibition)	Gastric (% inhibition)	Duodenal (% inhibition)
O1	58.6 ± 3.6	62.5 ± 4.1	66.9 ± 5.4	23.5 ± 3.5	28.5 ± 3.5	24.1 ± 3.8
O2	56.8 ± 5.8	64.8 ± 5.2	65.8 ± 5.5	22.9 ± 3.1	26.1 ± 2.4	26.5 ± 3.2
O3	64.9 ± 5.4	61.2 ± 5.3	63.2 ± 6.8	34.8 ± 2.9	39.4 ± 3.8	25.3 ± 3.5
O4	65.8 ± 3.9	59.8 ± 5.1	66.7 ± 6.7	33.6 ± 3.6	35.8 ± 3.4	31.2 ± 4.1
O5	56.4 ± 5.8	55.4 ± 5.2	58.4 ± 6.4	34.1 ± 4.2	37.6 ± 4.8	29.5 ± 4.0
O6	58.6 ± 6.4	56.3 ± 6.3	59.6 ± 6.8	33.9 ± 4.1	35.9 ± 4.2	38.5 ± 3.7
M1	66.5 ± 6.1	66.9 ± 6.2	68.9 ± 7.8	49.8 ± 3.8	50.9 ± 6.9	47.6 ± 3.8
M2	67.3 ± 5.8	66.4 ± 3.8	71.5 ± 6.9	47.2 ± 3.2	55.4 ± 7.1	49.2 ± 4.2
M3	69.7 ± 5.5	68.3 ± 3.4	72.8 ± 7.3	55.4 ± 2.8	60.3 ± 8.3	51.3 ± 4.1
M4	68.4 ± 6.1	62.5 ± 5.8	73.0 ± 8.1	56.7 ± 2.7	69.4 ± 8.6	58.4 ± 5.1
A1	79.8 ± 6.5	77.9 ± 5.9	82.5 ± 8.2	66.8 ± 4.9	70.3 ± 9.2	65.1 ± 6.8
A2	82.4 ± 3.8	83.0 ± 8.9	84.5 ± 6.8	64.9 ± 5.1	75.8 ± 9.1	66.2 ± 6.4
A3	84.1 ± 3.4	84.2 ± 8.8	86.5 ± 6.7	72.5 ± 6.4	86.0 ± 8.3	74.6 ± 6.9
A4	86.5 ± 3.8	85.9 ± 8.7	88.2 ± 8.3	73.1 ± 6.4	91.3 ± 9.3	72.9 ± 6.8
A5	88.2 ± 6.9	86.3 ± 6.9	81.4 ± 5.9	69.2 ± 5.8	91.3 ± 9.1	85.3 ± 8.2
G1	91.3 ± 5.4	92.6 ± 8.4	83.5 ± 5.2	85.9 ± 7.9	92.4 ± 8.6	81.2 ± 8.1
G2	90.3 ± 5.9	94.3 ± 8.5	85.9 ± 5.4	91.3 ± 8.1	90.1 ± 8.8	76.5 ± 7.3
Pi1	63.4 ± 6.8	61.4 ± 9.6	60.4 ± 6.8	34.5 ± 2.6	42.5 ± 5.3	32.4 ± 3.2
Li1	62.8 ± 6.1	62.3 ± 6.5	61.5 ± 6.4	21.9 ± 2.9	28.4 ± 3.4	21.9 ± 2.4
Le1	52.6 ± 5.4	54.2 ± 5.4	55.8 ± 5.9	25.9 ± 2.4	29.3 ± 3.2	28.3 ± 2.5
Pa1	51.8 ± 5.1	53.6 ± 5.8	55.4 ± 6.4	37.4 ± 3.6	40.9 ± 2.9	41.5 ± 8.9
W1	50.9 ± 4.9	51.8 ± 5.5	52.9 ± 6.3	29.6 ± 3.4	29.3 ± 2.8	28.6 ± 8.8

Values represent mean ± standard error mean of 3 or more independent repetitions

* $P < 0.05$, denotes statistically significant difference as compared with prior to in vitro digestion (ANOVA, Tukey's test)

the juices kept decreasing, indicating a better efficacy of the fruit juices to inhibit this enzyme. The α-glucosidase inhibitory activity of all juices increased statistically significantly ($P < 0.05$) following the duodenal phase of digestion, which was beneficial in terms of the therapeutic properties of the juices. In contrast, although the α-amylase inhibitory activities showed an opposite trend, the values could nevertheless be considered as high, indicating the ability of all fruit juices to regulate α-amylase enzyme activity even following digestion processes. Upon calculation of the regression (R^2) value of the total phenolics contents and α-amylase and α-glucosidase values prior to digestion as well as during the gastric and duodenal phases, it was observed that the R^2 values did not indicate an entirely linear response for any of the three phases (α-amylase: prior to digestion $R^2 = 0.75$, gastric $R^2 = 0.68$, duodenal $R^2 = 0.69$; (α-glucosidase: prior to digestion $R^2 = 0.77$, gastric $R^2 = 0.69$, duodenal $R^2 = 0.71$). Thus, it was concluded that the changes to the α-amylase and α-glucosidase values may not have been necessarily been due to changes in the total phenolics contents or their increased release.

4 Discussion

Public awareness has reached new heights to the extent that the term 'antioxidant' has been associated with therapeutic properties. Therefore, the marketing of many so-called 'superfoods' is commonly based on their antioxidant potential (Prior and Cao 2000). Although polyphenols are the most extensively studied group of antioxidants, non-phenolic compounds such as carotenoids and xanthins also contribute to the antioxidant capacity of food products. Thus, as a further study, it may be recommended that changes to the non-phenolic antioxidant compounds are also to be studied to have a more complete picture of the in vitro stability of the antioxidant capacity of food products. However, given the linear relationship between the ORAC values and the total phenolic contents of this study, it

Table 5 α-Amylase and α-glucosidase inhibitory activities of the fruit juices prior to digestion and following the gastric and duodenal phases as IC_{50}, micrograms Acarbose Equivalents per milliliter of sample (μg AE/mL)

Sample code	α-Amylase inhibitory activity			α-Glucosidase inhibitory activity		
	Prior (IC_{50}, μg AE/mL)	Gastric (IC_{50}, μg AE/mL)	Duodenal (IC_{50}, μg AE/mL)	Prior (IC_{50}, μg AE/mL)	Gastric (IC_{50}, μg AE/mL)	Duodenal (IC_{50}, μg AE/mL)
O1	78.9 ± 4.5	95.4 ± 7.8*	104.3 ± 12.3*	256.4 ± 20.5	236.8 ± 12.5	198.7 ± 9.8*
O2	68.7 ± 5.6	97.6 ± 8.5*	109.8 ± 13.1*	259.3 ± 19.5	218.9 ± 18.5	184.5 ± 8.6*
O3	69.3 ± 5.1	79.5 ± 9.1	88.6 ± 8.6*	264.9 ± 21.4	216.3 ± 10.9*	187.9 ± 11.9*
O4	98.3 ± 6.5	105.4 ± 10.1	126.7 ± 8.2*	248.9 ± 22.6	198.6 ± 21.5*	185.6 ± 9.7*
O5	73.2 ± 8.7	86.5 ± 6.3*	95.4 ± 7.6*	267.3 ± 31.9	218.9 ± 16.5*	184.6 ± 8.1*
O6	73.9 ± 6.9	89.4 ± 8.6*	96.1 ± 6.8*	284.1 ± 35.1	219.6 ± 24.6*	178.9 ± 9.1*
M1	105.9 ± 11.8	126.5 ± 9.7	138.7 ± 12.3*	369.8 ± 35.9	358.6 ± 12.5	297.8 ± 13.2*
M2	129.5 ± 12.8	156.4 ± 6.7*	179.4 ± 10.2*	379.9 ± 25.3	314.6 ± 11.5*	289.7 ± 10.9*
M3	139.8 ± 10.9	138.4 ± 11.3	156.3 ± 10.3	387.6 ± 31.5	318.5 ± 13.9*	257.6 ± 23.5*
M4	129.5 ± 12.8	132.3 ± 10.2	142.3 ± 8.7*	398.6 ± 33.4	358.9 ± 21.8*	258.4 ± 24.6*
A1	35.3 ± 4.5	25.3 ± 3.4	24.4 ± 3.2*	248.6 ± 46.9	198.6 ± 31.6*	179.5 ± 23.6*
A2	41.2 ± 6.1	31.2 ± 3.1	25.4 ± 2.7*	259.3 ± 31.9	214.6 ± 21.6	159.6 ± 21.6*
A3	32.9 ± 4.5	25.6 ± 4.7	21.5 ± 2.2*	261.7 ± 30.5	208.9 ± 16.9*	174.5 ± 16.8*
A4	30.3 ± 3.2	23.1 ± 1.9*	19.6 ± 1.9*	243.8 ± 31.2	210.6 ± 13.4	184.9 ± 15.2*
A5	31.8 ± 2.8	22.5 ± 1.2*	21.3 ± 1.8*	238.4 ± 29.5	198.6 ± 10.4	169.7 ± 16.9*
G1	22.3 ± 2.1	25.6 ± 3.5*	25.6 ± 2.3	201.6 ± 23.6	165.9 ± 12.8	135.8 ± 10.6*
G2	29.3 ± 2.2	30.2 ± 3.1	32.5 ± 2.6	198.3 ± 18.5	162.8 ± 11.5*	139.4 ± 11.5*
Pi1	84.3 ± 1.9	81.2 ± 3.4	79.6 ± 3.7	348.6 ± 22.6	308.6 ± 19.5	258.9 ± 24.9*
Li1	85.3 ± 6.8	80.3 ± 4.4	78.5 ± 4.3	259.6 ± 16.5	207.6 ± 16.6*	167.8 ± 18.9*
Le1	105.6 ± 9.5	115.6 ± 7.2	129.4 ± 7.8	248.3 ± 18.9	217.9 ± 14.3*	186.5 ± 17.4*
Pa1	127.6 ± 7.8	135.0 ± 12.2	143.6 ± 14.3*	357.6 ± 21.5	301.6 ± 13.8*	259.8 ± 19.2*
W1	36.5 ± 2.6	46.5 ± 1.9*	45.6 ± 2.1*	369.4 ± 23.6	325.7 ± 12.6*	264.9 ± 21.8*

Values represent mean ± standard error mean of 3 or more independent repetitions

* $P < 0.05$, denotes statistically significant difference as compared with prior to in vitro digestion (ANOVA, Tukey's test)

may be concluded that the primary source of antioxidant activity for the fruit juices were the phenolic compounds. The ORAC method is the most widely recognized assay used by food manufacturers despite its significant internal variability (Prior and Cao 2000). On the basis of technical issues related to temperature gradients across the plate in commonly used plate readers, this assay can have significant internal variabilities (Huang et al. 2005). Although this technical issue does not pertain to the end point determinations such as ABTS, FRAP and DPPH assays, the ORAC assay data was still included in this study for the overall determination of the TAC of the fruit juices. Due to this drawback, it is important to run multiple antioxidant assays rather than just the ORAC method to get a better estimate of antioxidant capacity. As for starch hydrolases, inhibition of α-amylase may deem comparatively more important when it comes to reducing the breakdown of starch, since it triggers the production of the substrate for the subsequent action of α-glucosidase. Given this requirement, even many of the commercially available anti-diabetic drugs to date, such as acarbose, primarily target the inhibition of α-amylase rather than α-glucosidase.

Previous studies detailing the bioaccessibility of the antioxidant capacity of commercially available fruit juices are sparse, although a few studies have been conducted on commercially available vegetable juices (Wootton-Beard et al. 2011; Ryan and Prescott 2010) and fresh fruit juices (Bermudez-Soto et al. 2007; McDougall et al. 2005). However, the stability of the starch hydrolase inhibitory activity of plant-based juices has not been studied to date. This study is also novel since no evaluations have been carried out to date on the fruit juices in commercially available Sri Lanka. The brands selected for this study differ from those studied previously by other researchers (Wootton-Beard et al. 2011; Borges et al. 2010). Given the increased consumer awareness, scientific data detailing the functional properties of commercial food products is beneficial for the local

consumers to make informed choices and to motivate the consumption of food products which have better therapeutic potentials. Preservatives with antioxidant effects were added to all fruit juices. In addition, there was a contribution of antioxidant activity from vitamin C abundantly present in all fruit juices. Citric acid had also been added to some of the juices which has been reported to account for up to 25 % of antioxidant activity but is not classified as a true antioxidant. It is difficult to quantify the specific contributions of these organic acids to TAC because no information was available on the amount used for each juice.

5 Conclusions

This study has shown that all fruit juices are a significant source of antioxidants with starch hydrolase inhibitory properties. However, there was a wide variation in the TAC and the inhibitory activity of the starch hydrolases between different types of juice. Grape, apple and mango juices displayed the highest antioxidant capacities across all of the assays conducted. The grape and apple juices were also observed to have the highest abilities to inhibit α-amylase and α-glucosidase. From a consumers' point of view, this research provided the first measurement concerning the stability of commercial fruit juice antioxidants and starch hydrolase inhibitory properties in Sri Lanka following in vitro digestion, although similar researches have been carried out in various other countries. As an additional measure for future studies, it is important that further biologically relevant information is provided on the antioxidants and starch hydrolase inhibitors by contributing data concerning the bioaccessibility and bioavailability of the bioactive compounds in a human system.

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Conflict of interest The mention of product or brand names in this article are for the purposes of scientific study only and do not imply any endorsements or recommendations by the Institute of Fundamental Studies, Sri Lanka. The authors report no conflict of interest, financial or otherwise.

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