Antioxidant properties of fruit infused water

Ganguli P. Ariyawansa* and Rahini Ramanathan

School of Applied Sciences, Faculty of Engineering, Science and Technology, Nilai University, Negeri Sembilan, Malaysia.
*Corresponding author. Tel.: +601164085512; E-mail: ganguliariyawansa@gmail.com
ORCID: https://orcid.org/0000-0001-6859-1925

Abstract

Fruit infused (or detox) water has been shown to possess many health benefits including toxin removal, increased immune function, weight loss and hydration. The present study aimed to determine and compare the antioxidant properties and total phenolic content (TPC) of water infused with Valencia orange (*Citrus sinensis* 'Valencia') and Fuji apple (*Malus domestica*). Fruits, either cubed or sliced and either with or without peel were added to distilled water in the ration 1:3w/v. The antioxidant activity (1, 1-diphenyl-2- picrylhydrazyl- DPPH assay) and total phenolic content (Folin Ciocalteu assay) of the soaking distilled water were measured at 0.5, 1,2,4,6 and 8 hours of infusion. The antioxidant potential of water increased with infusion time and ranged from 21% to 62.6% for apples and 39.5% to 77.6%, for oranges while TPC increased from 60.78 to 111.36 µg GAE / ml and 193.41 to 264.97 µg GAE / ml sample respectively. Sliced fruits with peel exhibited the highest antioxidant activity and this was attributed to their high TPC values. The fruit residue however, possessed low antioxidant and TPC values. The study has shown that fruit infused water possesses detoxifying effects due to the antioxidant potential of the polyphenolic content of the fruit. The study concludes that longer infusion time increases the antioxidant potential.

Keywords: DPPH assay, infused water, phenolic content

1. INTRODUCTION

1.1 Nature and importance of the study

Fruit infused water is a fusion of different fruits immersed in water, which can be customised based on the preferences of each individual. It is an alternate way to consume fruits with the intention of receiving the same health benefits and nutrition of the original fruit (Theola, 2018). Previous studies have shown that the nutritional benefits of consuming fruits are mostly due to the high phenolic (flavones,

anthocyanins, isoflavones) content and presence of other antioxidants such as carotenoids, tocotrienols, tocopherols and ascorbic acid (Thiagarajah et al., 2019).

Antioxidants prevent oxidative damage of lipids, proteins and nucleic acids by scavenging free radicals, thereby inhibiting the harmful effects caused by these compounds. Antioxidants have been proven to lower the occurrence of degenerative diseases such as cancer, arthritis, atherosclerosis, inflammation, brain dysfunction and they also slow down the aging process (Silva and Sirasa, 2018). Due to these numerous health benefits, investigations into analysing the antioxidant capacity of various fruits has generated a lot of interest recently (Metussin et al., 2017).

A recent study on the consumption of Jujube fruit leaf infusion as a bedtime beverage found that the leaf extract comprised of active ingredients that induce sleep by soothing the nerves (Yazdanpanah et al., 2017). Another study was carried out to evaluate the antioxidant activity of banana peel infused water, according to different infusion durations and water temperatures (Wassalwa and Rahmatan, 2016). Theola (2018) examined how the antioxidant activity fluctuated with different immersion durations in strawberry infused water. It was revealed in a recent study that herbal infusion of feijo had the same total antioxidant activity as the fresh fruit (Kabiri et al., 2016).

The significance of fruit infused water in elevating blood glucose level (prevention of hypoglycemic conditions) was verified in a study wherein it was shown that fruit infused water increased blood glucose in the human experimental group within the first 15 minutes of consumption and the elevated levels were well maintained within the second 15 minutes of consumption (Ali et al., 2016). Thiagarajah and colleagues (2019) explored the importance of fruit infused water as a fluid to be consumed during exercise. This study was carried out with 3 experimental groups, Fasting- Exercise only (FEO), Fasting- Exercise Water (FEW) and Fasting- Exercise with fruit infused water (FEF). Upon completion of the exercise routine, the dehydration level of each group was measured in terms of their urine colour. It was observed that the urine colour of the FEF group did not reach the dehydration level.

Even though the antioxidant properties of fruit pulp, leaves and fruit peels have been studied in previous research using various assays (total flavonoids content, total phenolic content, DPPH assay and ferric reducing antioxidant power assay), only a few studies have been conducted to evaluate the antioxidant properties of fruit infused water. Furthermore, previous studies have been conducted based on individual fruits. No comparison has been made between the antioxidant potential of water infused with different fruits. Further research is needed to determine whether the antioxidant potential in infused water matches the original quantity available in the fruit or declines upon infusion duration (Theola, 2018). At present, only a few studies on the health benefits of the modern trend of consuming fruit infused water are available. Besides that, investigations into the antioxidant potential of different fruits from previous studies have not compared fruits with and without peel.

Further research on the antioxidant properties of fruit infused water would help researchers to justify the potential benefits of fruit infused water rather than as merely a dietary trend.

2. METHODOLOGY

2.1 Preparation of fruit infused water

The fruits: Valencia orange (*Citrus sinensis* 'Valencia') and Fuji apple (*Malus domestica*) were purchased from supermarkets in Nilai, Negeri Sembilan. The fruits were washed under running water and rinsed with distilled water at room temperature (25 ± 1 °C) for thirty seconds to remove any contaminants present on the peels. The fruits were then patted dry with a clean towel to remove excess water. Two glass bottles with a maximum volume of 900ml were prepared for each fruit, one bottle containing fruit with peel and the other containing the same fruit without peel (Webber, 2018). Some trials were carried out by immersing cubed fruits in water while in other trials the fruits were sliced. A mandoline slicer was used to cut the fruits into uniform slices.

The fruits and water were prepared in 1:3 (weight: volume) ratio. The bottles were then closed firmly and left for 8 hours at room temperature (25 ± 1 °C) (Kooienga, 2013). Water samples were taken from the bottles at the specified time intervals of 0.5, 1, 2, 4, 6, and 8 hours.

2.2 Antioxidant assays

2.2.1 DPPH assay

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity was determined by the method described by Sridhar and Charles, (2019). An aliquot of 0.70 ml of fruit infused water (sample) was removed from each bottle at time intervals of 30 minutes, 1, 2, 4, 6 and 8 hours after infusion. After that, 0.70ml of (100μM) DPPH methanolic solution was added to 0.70ml of sample and mixed using a vortex mixer. The mixture was then incubated for 20 minutes at room temperature (25 ± 1 °C) in the dark. A UV/Vis spectrophotometer (Secomam Prim, RS232) was used to measure the decline in absorbance at 515 nm. The absorbance was measured against a blank of distilled water. Ascorbic acid (1mg/ml) was used as the positive control whereas a mixture of distilled water and DPPH was used as the negative control. The absorbance of both the control and the samples were recorded.

The scavenging effect of DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \left\{ \frac{A_{control} - A_{sample}}{A_{control}} \right\} \times 100$$

where, $A_{control}$ is the absorbance of negative control (distilled water + DPPH) and A_{sample} is the absorbance of the sample (fruit infused water) (Sridhar and Charles, 2019).

2.3 Total Phenolic Content (TPC) Assay

To determine the total phenolic content (TPC), the methodology of Arruda et al., (2016) was used with slight modifications. An aliquot of 0.10 ml (100 μL) of fruit infused water was removed from each bottle at time intervals of 30 minutes, 1, 2, 4, 6 and 8 hours after infusion. The TPC content of the fruit infused water was determined using the Folin-Ciocalteu method. Briefly, 0.10 ml (100 μL) of infused water sample was mixed with 100 μL of Folin-Ciocalteu reagent (50 % v/v) and 800 μL of sodium carbonate (5

% m/v). The solution was mixed using a vortex mixer and incubated for 20 min at 40 °C in a water bath (J-Lab Tech, LWB-1220). The absorbance was measured at 760 nm against a blank by using a UV/Vis spectrophotometer (Secomam Prim, RS232). The blank was prepared by replacing the sample with distilled water. Gallic acid in the concentration range of 0.5–8 μg/mL was used to plot the calibration curve. Average and standard deviations were calculated from triplicate measurements. The total phenolic content of each sample was expressed as μg Gallic Acid Equivalent per ml sample (μg GAE / ml sample) (Arruda et al., 2016).

2.4 Determination of antioxidant potential and phenolic content of left-over fruit slices/cubes

The measurement of antioxidant potential and phenolic content of left-over fruit slices/cubes was performed according to the method used by Sun et al., 2015. After 8 hours of infusion, the left-over fruit slices/cubes in each bottle were removed. The fruit slices/cubes were placed on glass petri plates and oven (Jeio Tech, Lab companion OF-11E) dried at 60°C to remove the moisture. The weight of the plates was taken at intervals and finally removed when a constant weight was recorded. The dried samples were powdered using a blender. Ten grams of each powdered sample (with peel and without peel) was put separately into conical flasks. After that, hundred milliliters (100ml) of 75% ethanol: water (4:1 v/v) solvent was added to each flask (Sun et al., 2015). The mixtures were then agitated using an orbital shaker (Protech, 720) for 60 min in the dark at room temperature (25 ± 1 °C). In order to remove the suspended particles, the homogenate was decanted and centrifuged at 3000×g for 15 minutes. Aliquots of the supernatant were taken from each centrifuged sample and DPPH and TPC assays were carried out as per the methods mentioned above, to determine the antioxidant potential and phenolic content (of left-over fruit residue) respectively. The supernatant was diluted 10-fold for DPPH assay and 50-fold for TPC assay.

2.5 Statistical analysis

Three replicate analysis was carried out for each sample. Statistical comparisons were made using T-test paired two sample for means. The statistical significance was set up at $p<0.05$ and all data were expressed as mean± standard deviation where n=3.

3. RESULTS AND DISCUSSION

Fig. 1 shows the percentage scavenging of water infused with Fuji apple (*Malus domestica*) cubes and slices. Samples were assayed in triplicates (n=3) in one biological trial whereas % scavenging at each time interval was calculated from the average absorbance at 515 nm. Vit. C (1mg/ml) was used as a positive control and exerted scavenging of 93.44%. After 0.5 hours of infusion, infused water containing apple cubes with peel had a lower percentage scavenging (65.67 %) than the infused water containing apple cubes without peel (84.64%). The percentage scavenging potential of infused water containing apple cubes with peel reached a maximum at 2 hours of infusion whereas the percentage scavenging potential of infused water containing apple cubes without peel reached a maximum at 0.5 hours, with values of 86.35% and 84.64 % respectively. The percentage scavenging of water infused with apple slices increased with the duration of infusion, for both samples with and without peel. Apple slices with and without peel exhibited 62.61% and 57.50% scavenging respectively after 8 hours of infusion. Infused water containing apple slices and cubes with peel displayed higher percentage scavenging activity than the infused water samples without peel after eight hours of immersion. The results are substantiated by a study by Wolfe et al (2003) who proved that apple peels possess a high content of phenolic compounds, antioxidant activity, and antiproliferative activity and thus should be regarded as a valuable source of antioxidants.

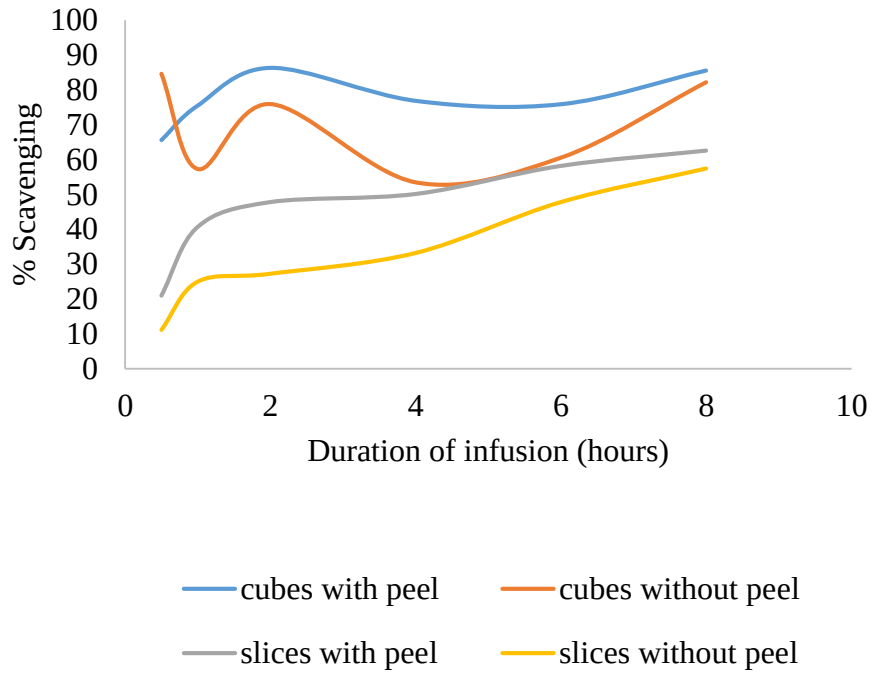

Fig.1. Percentage scavenging of water infused with Fuji apple (*Malus domestica*) cubes and slices (with and without peel)

Fig. 2 shows the percentage scavenging of water infused with Valencia orange (*Citrus sinensis* 'Valencia') cubes and slices. All samples were conducted in triplicates (n=3). Percentage scavenging of water infused with orange slices was calculated from the average absorbance readings. Vit. C (1mg/ml) was used as a positive control and exerted % scavenging of 62.35%. The percentage scavenging of orange infused water increased with the duration of infusion, for both cubed and sliced samples with and without peel. However, infused water containing orange with peel had higher percentage scavenging than the samples without peel. Infused water containing orange cubes with and without peel exhibited percentage scavenging of 77.15% and 73.89% respectively after 8 hours of infusion. Infused water containing orange slices with and without peel displayed percentage scavenging of 81.50% and 75.18% respectively after 8 hours of infusion. A study by Park et al (2014) has shown that orange peel does possess high antioxidant activity. The results thus indicate that fruits (apples and oranges) with peel possess higher antioxidant effects that fruits without peel.

On comparing the apple and orange sliced samples, the orange samples appeared to possess higher free radical scavenging activity compared to the apple samples with

values of 81.50% and 62.61% respectively. The results correlate to the findings of a study by Miller and Rice-Evans (1997), who showed that orange juice possessed higher phenolic antioxidants than apple juice.

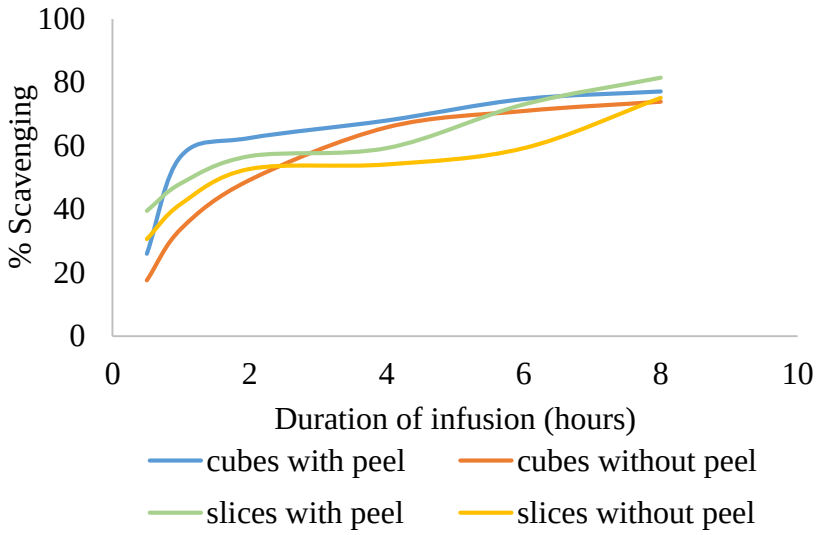

Fig. 2. Percentage scavenging of water infused with Valencia orange (*Citrus sinensis* 'Valencia') cubes and slices (with and without peel)

As can be seen from Fig. 3 the total phenolic content of all samples increased with infusion time. Samples (cubed and sliced) with peel possessed higher TPC values compared to samples without peel. The total phenolic content of infused water containing apple cubes with and without peel was 73.22 µg GAE / ml sample and 48.91 µg GAE /ml sample respectively after 8 hours of infusion. Infused water containing apple slices with and without peel possessed total phenolic content of 111.36 µg GAE/ ml sample and 100.57 µg GAE / ml sample respectively, after 8 hours of infusion. The total phenolic content for infused water with apple slices was greater than for infused water prepared with apple cubes.

From Fig. 1 above, it was shown that the samples with peel exhibited higher % scavenging activity than samples without peel. The samples with peel also contained higher TPC values than the sample without peel (Fig. 3). Thus, it can be assumed that the higher phenolic content contributed to the higher antioxidant activity of samples containing peel. The longer the fruits were infused in the water, the more phenolic compounds were extracted, leading to greater % scavenging of the infused water sample.

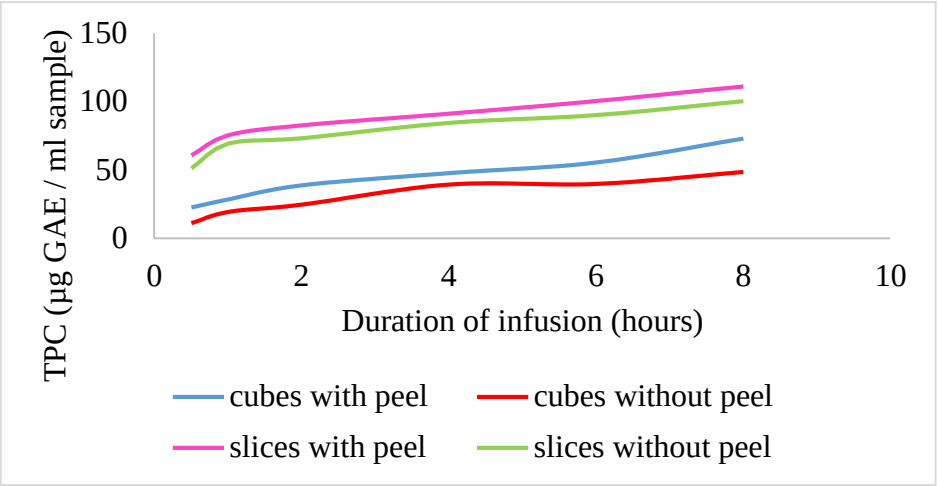

Fig. 3. TPC of water infused with Fuji apple (*Malus domestica*) cubes and slices (with and without peel)

Fig. 4 shows the total phenolic content of water infused with Valencia orange (*Citrus sinensis* 'Valencia') cubes and slices. Total phenolic content of orange infused water gradually increased with infusion time for samples (cubed and sliced) with and without peel samples. The infused water containing orange samples with peel showed a higher total phenolic content compared to water infused with samples without peel. Infused water containing orange slice with peel had the highest total phenolic content of 264.97 µg GAE / ml sample compared to orange slices without peel (163.38 µg GAE / ml sample), after 8 hours of infusion. The TPC value for infused water containing orange cubes with and without peel was 198.91 µg GAE / ml sample and 102.79 µg GAE / ml sample respectively, after 8 hours of infusion. Orange slices contained higher TPC values compare orange cubes. This correlates with the scavenging data obtained in Fig. 2 above.

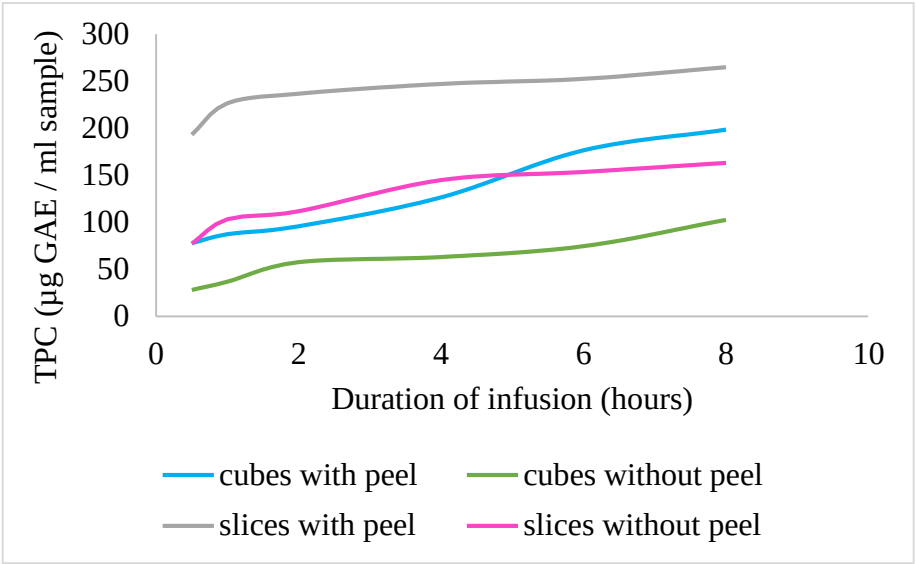

Fig. 4. TPC of water infused with Valencia orange (*Citrus sinensis* 'Valencia') cubes and slices (with and without peel)

Table 1 compares the % scavenging of fruit samples in cubed and sliced form at the eight hour infusion time. Letters in superscript indicate significance, with capital letters comparing the values in the rows and lowercase letters comparing values in columns. Comparison was made within the same fruit samples (cubes vs sliced and with peel vs without peel) and not between fruit samples. Apple and orange infused water containing peel demonstrated significantly higher percentage scavenging than the samples without peel. The water infused with apple cubes demonstrated a significantly higher percentage scavenging than the water infused with apple slices with values of 85.5 % vs 62.6 % for samples with peel and 82.23% vs 57.37% for samples without peel. The percentage scavenging of water infused with orange slices with peel (81.57%) was higher than the samples containing orange cubes with peel (79.01%), however the difference was not significant ($p<0.05$) as indicated by the same superscript "e".

Table 1. Comparison of % scavenging of water samples infused with sliced fruits vs cubed fruits at 8-hour immersion time.

% Scavenging of Fuji apple infused water		
	With peel	Without peel
Apple cubes	85.50 ± 0.77^{Aa}	82.23 ± 0.87^{Bc}
Apple slices	62.60 ± 1.13^{Cb}	57.37 ± 0.42^{Dd}
% Scavenging of Valencia orange infused water		
	With peel	Without peel
Orange cubes	79.01 ± 0.48^{Ee}	73.82 ± 0.30^{Ff}
Orange slices	81.57 ± 1.07^{Ge}	75.19 ± 0.06^{Hg}

The results indicate that the manner in which the fruits are cut is important when using apples, with cubed being better than the sliced. However, with regard to oranges, both the cubed and sliced form possess similar antioxidant potential. In general, fruits with peel appear to possess higher % scavenging than fruits without peel.

Table 2 compares the percentage scavenging of the left-over Fuji apple and Valencia orange residues. Letters in superscript indicate significance, with capital letters comparing the values in the rows and lowercase letters comparing values in columns. Comparison was made within the same fruit samples (cubes vs sliced and with peel vs without peel) and not between fruit samples. Residues with peel showed significantly higher percentage scavenging than the residues without peel except for the orange cubed samples. A significantly greater ($p< 0.05$) percentage scavenging was shown only by apple slices, compared to apple cubes with values of 5.56 vs 2.16 µg GAE/ml sample respectively for samples without peel.

Table 2. Comparison of percentage scavenging of the residues of the sliced vs cubed fruits at 8-hour infusion time

% Scavenging of Fuji apple residue		
	With peel	Without peel
Apple cube	7.54 ± 0.55^{Aa}	2.16 ± 0.48^{Bb}
Apple slice	9.61 ± 0.77^{Ca}	5.56 ± 0.91^{Dc}
% Scavenging of Valencia orange residue		
	With peel	Without peel
Orange cube	15.86 ± 2.05^{Ed}	8.26 ± 5.30^{Ee}
Orange slice	19.19 ± 2.51^{Fd}	10.58 ± 2.96^{Ge}

Table 3 compares the total phenolic content of water infused with Fuji apples and Valencia oranges. Letters in superscript indicate significance, with capital letters comparing the values in the rows and lowercase letters comparing values in columns. Comparison was made within the same fruit samples (cubes vs sliced and with peel vs without peel) and not between fruit samples. The water infused with apple cubes, orange cubes and orange slices with peel (containing 73.28, 198.93 and 264.98 µg GAE/ml sample respectively) demonstrated significantly higher ($p< 0.05$) total phenolic content than the samples without peel (containing 48.92, 102.98 and 163.42 µg GAE/ml sample). With apple slice however, the samples with and without peels possessed similar TPC values, as indicated by the same superscript letter "C". Water infused with sliced fruits showed significantly higher ($p< 0.05$) total phenolic content than the water infused with cubed fruits. For apples, the values were 111.38 (slice) vs 73.28 (cube) µg GAE/ml sample for samples with peel and 100.58 (slice) vs 48.92 (cube) µg GAE/ml sample for samples without peel. For oranges the values were 264.97 (slice) vs 198.91 (cube) µg GAE/ml sample for samples with peel and 163.38 (slice) vs 102.79 (cube) µg GAE/ml sample for samples without peel.

Table 3. Comparison of the total phenolic content of water infused with sliced fruits vs cubed fruits at 8 hours

TPC of Fuji apple infused water (µg GAE/ml sample)		
	With peel	Without peel
Apple cube	73.28 ± 0.82^{Aa}	48.92 ± 0.57^{Bc}
Apple slice	111.38 ± 2.55^{Cb}	100.58 ± 5.89^{Cd}
TPC of Valencia orange infused water (µg GAE/ml sample)		
	With peel	Without peel
Orange cube	198.93 ± 1.34^{De}	102.83 ± 3.26^{Eg}
Orange slice	264.98 ± 0.70^{Ff}	163.41 ± 2.72^{Gh}

Table 4 compares the total phenolic content of Fuji apple and Valencia orange residues. Letters in superscript indicate significance, with capital letters comparing the values in the rows and lowercase letters comparing values in columns. Comparison was made

within the same fruit samples (cubes vs sliced and with peel vs without peel) and not between fruit samples. No significant difference ($p < 0.05$) was observed in the TPC of apple residue, in terms of the presence/absence of peel and the surface area of the residue (cubes/slices) as indicated by the same superscript letters. However, orange residues with peel exhibited significantly higher TPC than the samples without peel, with values of 19.90 vs 11.46 µg GAE/ml sample respectively for cube samples and 41.01 vs 25.33 µg GAE/ml sample respectively for sliced samples. Orange slices showed significantly higher ($p < 0.05$) TPC, compared to orange cubes as indicated by different superscript lowercase letters. The values are more than doubled.

Table 4. Comparison of the total phenolic content of residues of the sliced vs cubed fruits at 8 hours

TPC of Fuji apple residue (µg GAE/ml sample)		
	With peel	Without peel
Apple cube	8.44 ± 2.08^{Aa}	6.03 ± 4.17^{Ab}
Apple slice	15.68 ± 2.09^{Ba}	10.85 ± 3.62^{Bb}
TPC of Valencia orange residue (µg GAE/ml sample)		
	With peel	Without peel
Orange cube	19.90 ± 1.81^{Cc}	11.46 ± 2.76^{De}
Orange slice	41.01 ± 4.18^{Ed}	25.33 ± 3.62^{Ff}

As per the results obtained, the longer the duration of infusion, the greater the percentage scavenging and total phenolic content. This is supported by Castiglioni et al. (2015) wherein it was shown that, when some white and green teas were steeped at room temperature for different time durations (15, 30, 60, 120 min), the total phenolic content, total flavonoid content and antioxidant activity of the samples increased with increasing time (120 > 60 > 30 > 15 min). Similarly, İlyasoglu and Arpa (2017) reported that diffusion of antioxidants was influenced by the infusion time and concluded that longer infusion times resulted in increased antioxidant extraction up to the maximum level. According to Kyle et al. (2007) the TPC of black tea beverage increased when the infusion time was increased from 3 to 10 minutes. Moreover, according to Kelebek (2016), when black tea was infused in water for 3,6 and 10 minutes, the highest TPC was observed at the highest infusion time. Yang et al. (2007) justified this considering that at room temperature the bioactive compounds require adequate time to diffuse from its source material into water.

According to the results obtained from this study, in general, the percent scavenging and TPC were higher in water infused with fruit slices compared to water infused with fruit cubes. In a previous study it was found out that, grinding tea leaves increased the surface area of the leaves that was contacted with the solvent, hence eased the passage of polyphenols and antioxidants from the leaves to the solvent (water) (Castiglioni et al., 2015). This was further confirmed by Chaturvedula and Prakash (2011), wherein it was observed that the tea prepared with ground leaves contained higher amounts of

phytochemicals compared to the tea prepared with whole leaves. Since the fruit slices have a larger surface area compared to fruit cubes, it is not surprising that the percent scavenging and TPC were higher in water infused with fruit slices compared to water infused with fruit cubes.

Based on the results, it was observed that, high TPC resulted in high percentage scavenging. This is because, the polyphenols available in plants also have antioxidant properties (Heng et al., 2017; Thiagarajah et al., 2019). This is supported by the study of Sim et al. (2010) wherein it was shown that the capacity of antioxidant was greatly associated with the total phenolic content of the plant leaves' crude extract. Likewise, Yao et al. (2010) stated that there was a positive correlation between the antioxidant activity and the total phenolics content in celery. Another study also revealed that the high antioxidant activity of *G. bursa - pastoris* extracts was due to the presence of high phenolic content (Ramdani et al., 2017).

As per the results obtained, infused water containing fruits with peel had a higher percentage scavenging and TPC compared to the infused water containing fruits without peel. Likewise, the percentage scavenging and TPC were higher in fruit residues with peel, compared to ones without peel. These results are supported by Lim et al. (2007) wherein it was witnessed that non-peeled guava fruits demonstrated higher antioxidant contents than peeled guava fruits. This is because the fruit peels are comprised of polyphenolic phytochemicals that have antioxidant properties. Hence, once fruits are infused in water with its peel, these phytochemicals diffuse from peel into the water, resulting in higher percentage scavenging and TPC in the infused water (Kubola and Siriamornpun, 2011).

It was observed that the percentage scavenging and TPC of fruit residues were lower than the percentage scavenging and TPC of infused water. This is because, the longer the duration of infusion, the more the phytochemicals diffused into the water from the fruit (Castiglioni et al., 2015). Hence prolonged durations of infusion caused most of the bioactive compounds present in the fruits to be released into water, which resulted in lower TPC and antioxidant potential in the left-over fruit residues.

4. CONCLUSION

In conclusion, prolonged infusion caused the infused water to have higher percentage scavenging and total phenolic content. The peels of oranges contain a phenolic compound called hesperidin in large amounts whereas apple peels have a greater total phenolic content than the flesh. Thus, the infused water containing fruits with peel demonstrated higher total phenolic content than the infused water containing fruits without peel. These phenolics in turn greatly contributed to the antioxidant potential of the fruit. It can be concluded that removing the peel results in a significant loss of

phenolics and antioxidants. Furthermore, infused water prepared with fruit slices exhibited higher antioxidant capacity and TPC, compared to infused water prepared with fruit cubes. The findings of the study would serve as a set of proven data to promote the benefits of consuming fruit infused water for people suffering from degenerative diseases, hypoglycemic conditions, sleep disorders, dehydration, etc. Further studies can be made to determine the antioxidant properties of fruit infused water by using different antioxidant assays such as FRAP and ABTS.

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