

Investigation into the Total Phenols and Antioxidant Activity during Storage of Fruit Smoothies

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Abstract: In this study, the total phenols (TP) and antioxidant activity of three different fruit smoothies (Blackberries, Raspberry and Boysenberry (BRAB), Mango and Passion fruits (MAP) and Blackcurrant, Acerola cherries and Rosehips (BACAR)) were determined during storage by Folin-Ciocalteu and ferric reducing antioxidant power (FRAP) assays respectively. TP ranged from 1,943 to 2,692 mg L⁻¹ gallic acid equivalent whereas FRAP from 43,217 to 126,125 μ mol L⁻¹ Trolox equivalent for the three smoothies on opening. BACAR had the highest TP and antioxidant activity. The smoothies showed significant decrease ($P < 0.05$) in TP during storage. The decrease in antioxidant activity was significant ($P < 0.05$) for BRAB and MAP only. However, the study revealed that the smoothies had high antioxidant activity even during shelf-life at 4 °C which implies that consumption of smoothies may give considerable antioxidant benefits. Correlation between TP and antioxidant activity was strong and highly significant ($r = 0.890$, $P < 0.0001$).

Key words: Total phenols, antioxidant activity, fruit smoothies, storage.

1. Introduction

The process of oxidation in the human body generates free radicals that can damage membranes and other structures including cellular proteins, lipids and DNA. Dietary antioxidants are substances obtained from the diet which participate in physiological, biochemical and cellular processes. They inactivate free radicals and delay or prevent free radical initiated chemical reactions when present in low concentration with those of an oxidizable substrate [1]. Recently, phenols in foods have gained much attention owing to their antioxidant activity and possible beneficial implications in human health such as prevention of certain cancers and cardiovascular diseases. Many fruit juices contain certain phenolic compounds which possess antioxidant capacity. Phenolics account for the major contribution to antioxidant potential of non-citrus juices while vitamin C accounts for about 65%-100% of juices derived from citrus fruits [1, 2]. Other studies had shown that polyphenols account for

the majority of antioxidant activity of many fruits when compared to ascorbic acid and other antioxidants [3, 4]. However, polyphenols can undergo various reactions in the course of food processing and storage. They serve as substrates for the browning enzymes (polyphenol oxidases or phenoloxidases) and enzymes such as esterases, glycosidases and decarboxylases catalyse the transformation and degradation reactions of polyphenolic compounds [5, 6]. Processing and prolong storage can also promote or enhance enzymatic or chemical oxidation of phenolic compounds. Stability of polyphenols is also influenced by pH [7] and properties of polyphenols are greatly affected by their interaction with the food matrix, such as in the presence of sugars and ascorbic acid [1, 6]. Phenolics can be affected by storage conditions such as temperature, atmosphere and light. Oxygen has been reported to be an important factor in destabilizing polyphenols, especially anthocyanins, and antioxidant capacity of processed products [8].

A fruit smoothie is described as a 100% blend of a variety of fruits with no added sugar or additives and

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not made from concentrates. Smoothies are new fruit products of increasing emergence in the market [9]. Smoothies may have increased nutritional and health benefits over fruit juices since they are closer to the whole fruits, thus they contain the flesh, pulp and fibre of the fruits as they are made from the whole (edible) crushed fruits. Smoothies mainly contain highly coloured fruits rich in antioxidants that are linked to providing protection against several diseases. Commonly used fruits include berries which are rich sources of flavonoids (such as anthocyanins, proanthocyanins, flavonols and catechins representing the main subgroups) and phenolic acids as well as antioxidant vitamins. Citrus fruits contain substantial level of flavonone glycosides. Orange juice contains predominantly naringin along with other neohesperidosides. Grapes contain large amount of polyphenols, majority being caftaric acid, tartaric acid ester of caffeic acid, flavan-3-ol catechin and blue-red pigment malvidin-3-glucoside, the major anthocyanin. Apples are also rich sources of phenolic acids and flavonoids. Mango peel and pulp also contain considerable antioxidants and polyphenols such as quercetin, kaempferol, gallic acid, caffeic acid, catechins, tannins and the unique mango xanthone, mangiferin [1, 10-12].

Previous studies on fruits and juices indicated that phenols content may increase [13], decrease [14-16] or remain unchanged [13, 17] during storage. Some studies had also shown that vitamin C in fruits and juices decrease during storage which also influences the antioxidant activities [18-20]. However, the fate of polyphenols and antioxidant activity in smoothies during shelf-life has not been reported.

This study, therefore, aims to investigate the total phenols and antioxidant activity of fruit smoothies during storage.

2. Materials and Methods

2.1 Materials

Folin-Ciocalteu phenol reagent was purchased from

Fisher Chemicals (Loughborough, UK). 6-hydroxy-2, 5, 7, 8-tetramethyl chroman-2-carboxylic acid (Trolox) was purchased from Acros Organics (Loughborough, UK). Standard gallic acid, standard vitamin C and 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma-Aldrich (Poole, Dorset, UK). All chemicals used were of analytical grade.

Three different types of smoothie drinks namely; Blackberries, Raspberries and Boysenberries (BRAB), Mango and Passion fruit (MAP), and Blackcurrants, Acerola cherries and Rosehips (BACAR) were purchased from Innocent Ltd. (London, UK). Besides the major ingredients representing the bearing name, all the smoothies contained a similar quantity of apple and banana with either orange juice, lemon or lime juice. The ingredients composition of each fruit smoothie is as presented in Table 1. These drinks were selected in order to make comparisons based on their fruit ingredients. To ensure control of age and handling of samples prior to analysis, all smoothie varieties/batches were purchased on the same day when freshly made and the investigations were carried out within their use by/expiry dates.

2.2 Sample Preparations

The fruit smoothies were stored at 4 °C for 4 weeks unopened while some were opened, part sampled and analysed daily for 4 consecutive days. Some drinks were also stored at ambient temperature (21 ± 1 °C) for two days and sampled for analysis daily. Prior to total phenols and antioxidant activity determinations, each smoothie was diluted 10-fold and then centrifuged (Beckman Model TJ-6 centrifuge) at $2,000 \times g$ for 20 min.

2.3 Determination of Total Phenols

The total phenol content was analysed using an adapted Folin-Ciocalteu method [2, 21]. 0.1 mL of sample, standard or blank (distilled water) was pipetted into a 10 mL volumetric flask and 6.0 mL of distilled water added. This was followed by adding 0.5 mL of

Table 1 Ingredient compositions, vitamin C, mean total phenols content and antioxidant activity of fresh fruit smoothies.

Fruit smoothie	Ingredients	Vitamin C* (mg L ⁻¹)	Total phenols (mg L ⁻¹ , GAE) Mean ±SD	Antioxidant activity (µmol L ⁻¹ , TE) Mean ± SD
BRAB	47 crushed raspberries (15%)	100	2,040.0 ± 23.6 ^a	75,792 ± 3,359 ^a
	22 crushed boysenberries (10%)			
	20 crushed blackberries (8%)			
	8 pressed apples			
	2 mashed bananas			
MAP	17 pressed white grapes	240	1,943.3 ± 70.7 ^a	43,217 ± 1,225 ^b
	1 orange and a dash of lemon juice			
	2 ½ crushed Mangoes (23%)			
	2 crushed passion fruits (5%)			
	11 pressed apples			
BACAR	1 ½ mashed banana	910	2,692 ± 21.6 ^b	126,125 ± 2,887 ^c
	2 freshly squeezed oranges			
	a squeeze of lemon			
	450 blackcurrants (14%)			
	28 acerola cherries (11%)			
	6 rosehips (2%)			
	9 pressed apples			
	3 mashed bananas			
	1 freshly squeezed orange			

*Package labels. SD: Standard Deviation (n = 6). Means in each column with different letters are significantly different ($P < 0.05$). BRAB: Blackberries, Raspberries and Boysenberries; MAP: Mango and Passion fruit; BACAR: Blackcurrants, Acerola cherries and Rosehips. GAE: Gallic acid equivalent; TE: Trolox equivalent.

Folin-Ciocalteu reagent (2N). The solution was thoroughly mixed and left to stand for 5 min at ambient temperature. 1.5 mL of 20% Na₂CO₃ solution was added and the solution was made up to the 10 mL mark with distilled water and mixed thoroughly. The resulting solution was then incubated at ambient temperature for 2 h in the absence of light after which absorbance readings were taken at 765 nm using a spectrophotometer (Shimazu UV-VIS 160A). All measurements were performed at least in triplicate on six different cartons of each smoothie. A calibration curve was constructed using standard gallic acid and the results expressed as gallic acid equivalent (GAE, mg L⁻¹).

2.4 Determination of Antioxidant Activity

The antioxidant activity was determined by using the ferric reducing antioxidant power (FRAP) assay [2, 22, 23]. The FRAP reagent was prepared by mixing 200 mL of 300 mM acetate buffer, 20 mL of 10 mM TPTZ solution, 20 mL of 20 mM FeCl₃ solution and 24 mL distilled water. The FRAP reagent, TPTZ and FeCl₃ solutions were freshly prepared on each day of assay. A 0.2 mL of sample extract (diluted by 10%), standard or

blank (distilled water) was pipetted into a test tube and 6.0 mL of FRAP reagent added. It was mixed and the absorbance was read with a spectrophotometer (Shimazu UV-VIS 160A) at 593 nm after 10 min. All measurements were performed at least in triplicate on six different cartons of each smoothie. A calibration curve was constructed using standard Trolox and the results expressed as micromol Trolox equivalent (TE, µmol L⁻¹).

2.5 pH Measurement

In order to assess for any changes during storage and correlation with the antioxidant properties, the pH of all smoothies (both fresh and stored) was also measured using a Jenway 3305 pH meter.

2.6 Statistical Analysis

The results obtained were analysed statistically using SPSS version 12 (SPSS Inc., IL, USA). The mean total phenol content and antioxidant activity of smoothies at different storage times and conditions were compared using one-way ANOVA followed by LSD post hoc multiple comparisons.

3. Results and Discussion

3.1 Total Phenols Content and Antioxidant Activity of Fresh Smoothie Drinks

The total phenols (TP) and antioxidant activity of three different types of fruit smoothie were determined. BACAR smoothie had the highest TP, followed by BRAB smoothie and MAP smoothie (Table 1). The differences in TP could be attributed to the different fruit ingredients. Berries are known to have high levels of phenols; however, sour cherries in BACAR contain relatively higher TP as compared to berries [24]. Therefore, blackcurrants and cherries as well as the percentage composition of other fruits (apples, bananas, orange juice) could possibly account for the relatively high TP content of BACAR compared to MAP and BRAB drinks. Results showed that smoothies have a higher TP in comparison to some fruit juices reported previously. Piljac-Žegorac and others [25] monitored six industrial dark fruit juices and found that blackcurrant juice had the highest TP content (1,920 mg L⁻¹ GAE). Comparatively, smoothies had higher TP than that of various fruit juices reported by Gardner and others [2] with orange juice having the highest TP content at 755 mg L⁻¹ GAE. The higher TP content of the smoothies compared to these juices could be due to significant contribution from their various fruit ingredients. Furthermore, unlike juices, smoothies contain the pulp and the skins of some fruits thereby enriching the polyphenols content [24]. Vitamin C content was the highest in BACAR, followed by MAP and BRAB (Table 1). As with TP, BACAR had the highest antioxidant activity, followed by BRAB and lastly MAP (Table 1). However, MAP had higher vitamin C than BRAB but antioxidant activity of MAP was significantly lower than that of BRAB. This suggests that TP contributes largely to the antioxidant activity of smoothies in comparison to vitamin C. These findings are in agreement with previous reports that showed polyphenols account for the majority of antioxidant activity of many fruits when compared to

ascorbic acid and other antioxidants [3, 4, 14]. It has also been reported that phenolics have a major contribution to antioxidant potential of non-citrus juices while vitamin C was found to account for about 65%-100% of juices derived from citrus fruits [2]. Correlation between TP and antioxidant activity of smoothies was found to be highly significant ($r = 0.890$, $P < 0.0001$), which is in agreement with other findings [2, 14, 26, 27].

3.2 Total Phenols and Antioxidant Activity during Storage at 4 °C

Smoothies were stored unopened at 4 °C for 4 weeks and a general significant decrease was observed in TP (Fig. 1).

A possible reason for the observed decrease in TP during storage could be due to the polyphenols reacting with sugars and sugar metabolites present in the smoothies. For instance, stability of anthocyanins is known to decrease as sugar content increases [28]. At the end of the 4 weeks storage period, BACAR still had the highest TP content (2,173 mg L⁻¹ GAE), followed by BRAB (1265 mg L⁻¹ GAE) and lastly MAP (936 mg L⁻¹ GAE). MAP experienced more average loss in TP (46%-60%) than that of BRAB (28%-38%) and BACAR (14%-20%) after the first to fourth week of storage unopened. The variation in decreasing rate could be dependent on the type of polyphenols and their stability.

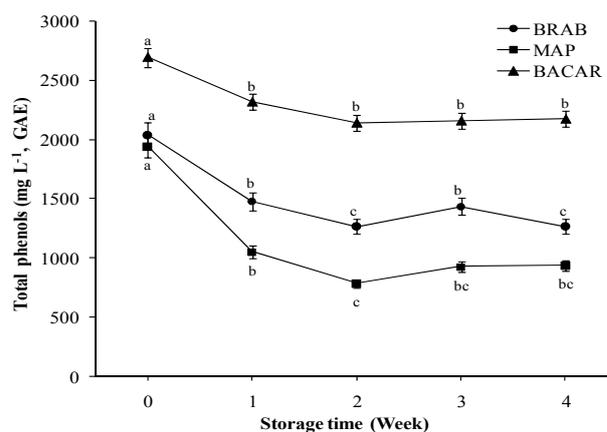


Fig. 1 Total phenol content of fruit smoothies during storage at 4 °C. GAE: Gallic acid equivalent. ^{a-c} Treatments on the same line (same smoothie) with different letters are significantly different ($P < 0.05$).

The pH of the smoothies could be another possible factor. The pH is thought to contribute significantly to the oxidative stability of total phenols, vitamin C and thus antioxidant activity of smoothies because low pH helps to inhibit microbial growth and some enzymatic reactions. The stability of polyphenols (particularly anthocyanins) is high at low pH [7]. Vitamin C is also stable at low pH and destroyed by increased pH [29]. Even though the pH of the smoothies was quite low (data not shown due to no significant variations; 3.2-3.4, 3.1-3.3 and 3.1-3.2 for MAP, BRAP and BACAR respectively) for both fresh and stored samples, stability of polyphenols was still affected. It was found that TP and antioxidant activity correlate significantly with pH in an inverse relationship ($r = -0.519$ and -0.534 respectively). However, pH of the smoothies was relatively stable after the first two weeks to the fourth week with that of BACAR being most stable.

In general, antioxidant activity of smoothies decreased during storage (Fig. 2) and the decrease could be attributed to the loss of TP. After one week of cold storage at 4 °C, the antioxidant activity of BRAB, MAP and BACAR were 87%, 82% and 91% respectively in comparison to their respective fresh samples. At the end of the fourth week of storage, MAP had approximately 77%, BRAB 82% and BACAR 93% antioxidant activity in comparison to the respective fresh samples. The decrease in antioxidant capacity for BACAR from fresh to week four was not significant while that of BRAB and MAP were significant ($P < 0.05$, Fig. 2). This may be due to the relatively high content and/or stability of vitamin C and polyphenols in BACAR, thereby maintaining its antioxidant activity.

Miller and Rice-Evans [30] suggested that the phenolic antioxidants protect vitamin C against oxidative decomposition with those in blackcurrants having the greatest vitamin C sparing activity than oranges and apples. Polyphenols may protect vitamin C by donating hydrogen to convert dehydroascorbic acid back to ascorbic acid in redox reactions.

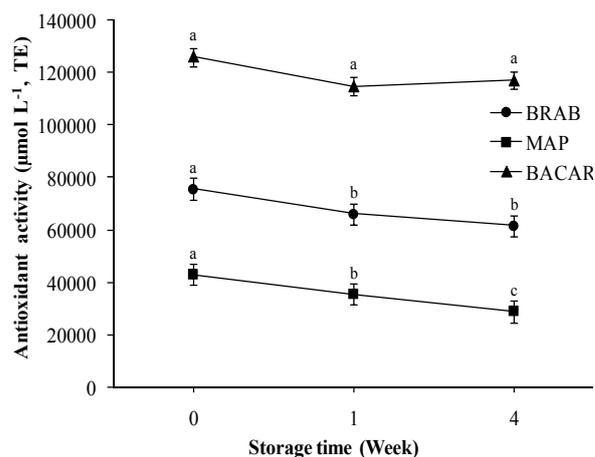


Fig. 2 Antioxidant activity of smoothies during storage at 4 °C. TE: Trolox equivalent. ^{a-c} Treatments on the same line with different letters are significantly different ($P < 0.05$).

There was a significant loss ($P < 0.05$) in TP and antioxidant activity over four days when smoothies were opened, part taken out and the remaining put back in the refrigerator (Fig. 3). This is as a result of oxygen entry into the container each time the carton was opened and consequently causing degradation of polyphenols and vitamin C [29]. The decrease continued with storage time possibly because of more oxygen entering into the container during re-opening. The variation in decrease may also depend on the volume of oxygen that entered the container. A similar effect has been observed where oxygen availability rate accounted for the highest effect on lowering antioxidant activity during pasteurization and storage of black chokeberry juice [15].

Introduction of oxygen when blueberry juice samples were shaken has also been reported to cause a significant negative effect on monomeric anthocyanins, total phenolics and antioxidant capacity [8]. From these observations, it is therefore important that once the smoothie is opened, it should be consumed within the stated four days and should be quickly closed to minimize oxygen entry if not all will be consumed.

3.3 Total Phenols and Antioxidant Activity during Storage at Ambient Temperature

Smoothies stored at ambient temperature (21 ± 1 °C) experienced loss in TP (Fig. 4a). A similar observation

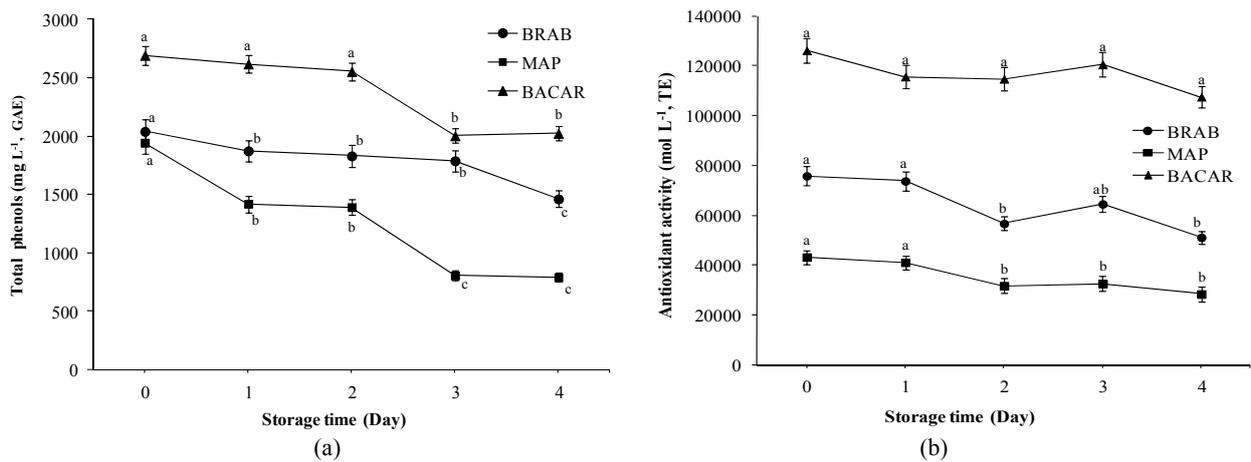


Fig. 3 (a) Total phenols and (b) antioxidant activity of smoothies when opened and refrigerated (at 4 °C) over four days. ^{a-c} Treatments on the same line with different letters are significantly different ($P < 0.05$).

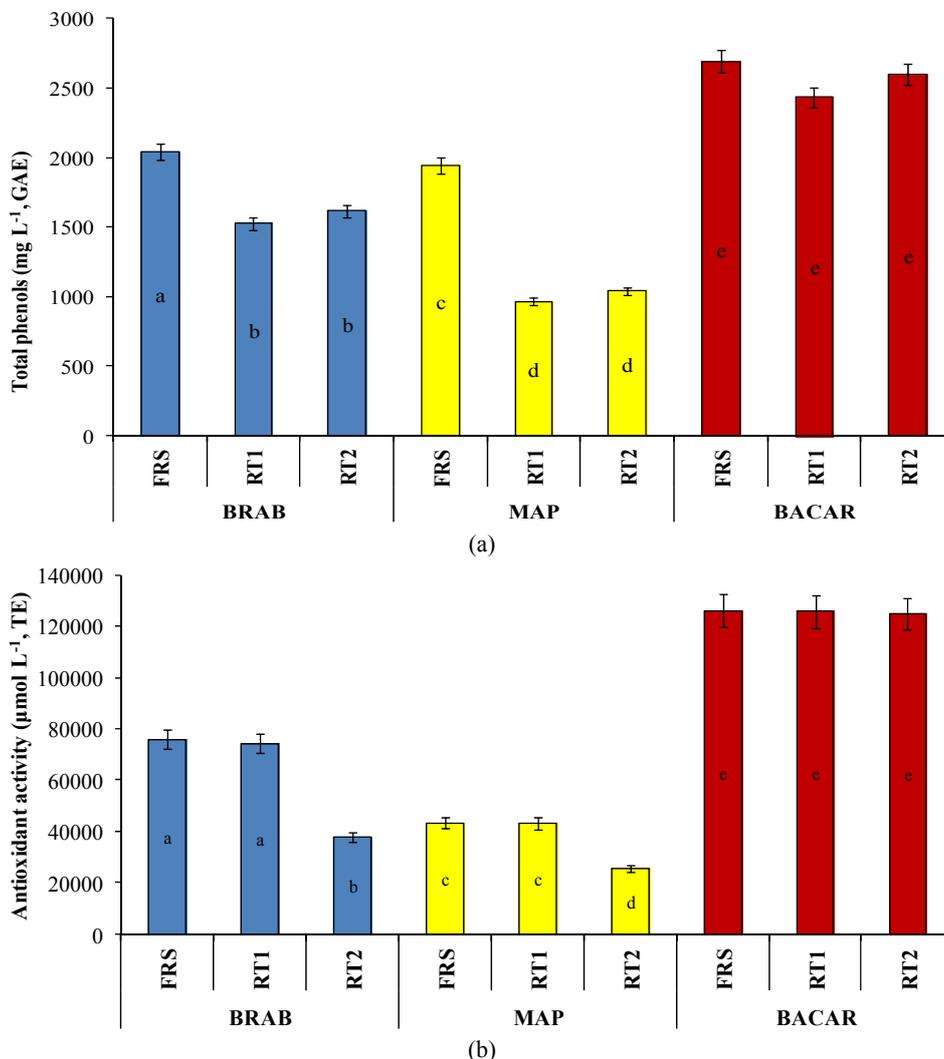


Fig. 4 (a) Total phenols and (b) antioxidant activity of smoothies at ambient temperature (21 ± 1 °C). GAE: Gallic acid equivalent; TE: Trolox equivalent. FRS: Fresh (Control) sample; RT1: Room Temperature Day 1; RT2: Room Temperature Day 2. ^{a-c} Treatments/Bars of the same colour (same smoothie) with different letters are significantly different ($P < 0.05$).

has been previously reported for apple juices [14]. BRAB had approximately 25% loss in TP, MAP 50% loss and BACAR had 10% loss when kept at ambient temperature over 24 h. The TP loss in BACAR was not significant but those of BRAB and MAP were significant ($P < 0.05$). The polyphenols in BACAR might be more stable or less susceptible to degradation at ambient temperature than those in BRAB and MAP. Similarly, the antioxidant activity of smoothies also decreased slightly when stored at ambient temperature (Fig. 4b). The decrease in antioxidant activity was not significant after the first day but highly significant after the second day for MAP and BRAB. Antioxidant activity of BACAR was almost the same even after the second day. Like MAP and BRAB, and unlike BACAR, antioxidants in *noni juice* have been reported to be unstable when stored at 24 °C [16]. Formation of slight colour changes and bubbling due to fermentation were observed in each smoothie by the first 24 h of storage at ambient temperature and this could be attributed to the observed decline in antioxidant properties especially for MAP and BRAB. Therefore it is important to avoid leaving the smoothies at ambient temperature so as to maintain the beneficial qualities.

4. Conclusion

In this investigation, different types of fruit smoothies were found to have different total phenols content and antioxidant activities, attributable to their respective fruit ingredients. There was a significant loss ($P < 0.05$) in TP and antioxidant activity over 4 days storage at 4 °C when smoothies were opened, part taken out and the remaining put back in the refrigerator. This is as a result of oxygen entry into the container which causes degradation of polyphenols and vitamin C. When smoothies were stored at ambient temperature the losses in TP, and antioxidant activity were variable. During unopened storage of the smoothies at 4 °C for 4 weeks, all smoothies experienced a significant decrease ($P <$

0.05) in TP. The decrease in antioxidant activity for BACAR was not significant while that of BRAB and MAP were significant. This study revealed that the smoothies used had high TP and antioxidant activity even after the four weeks shelf-life at 4 °C which implies that consumption of smoothies may give considerable antioxidant benefits.

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