mixtures of vanillic acid and caffeic acid were prepared at different ratios: (a) VA: Caff (1:1, v/v) and (b) (3:1, v/v). Both standards had concentrations of 0.01 mg/mL, and the injection volume was 20 µL. With the VA: Caff (1:1) mixture, 0.1 µg vanillic acid and 0.1 µg caffeic acid was injected; with VA: Caff (3:1) mixture, 0.15 µg vanillic acid and 0.05 µg caffeic acid was injected. VA: Caff (1:1) mixture was first injected, followed by VA: Caff (3:1) mixture. The area of peak 1 increased from 32% (Appendix 1, peak 1a) to 64% (peak 1b); area of peak 2 reduced from 68% (peak 2a) to 36% (peak 2b). Thus, it was deduced that peak 1 represented vanillic acid and peak 2 represented caffeic acid, and that vanillic acid eluted before caffeic acid.

Statistical analysis

Statistical analyses of all the results were carried out using one-way analysis of variance (ANOVA) to assess for any differences between the samples. The means obtained were separated by Tukey pairwise comparison test at 95% confidence level using MINITAB 17 statistical software (Minitab® Statistical Software, 2010). All data were presented as means ± SD (n=6). Pearson's coefficient (R^2) was also identified for all calibration curves. Pearson's correlation (r) was calculated at P<0.05 and 0.01 using the same software.

RESULTS AND DISCUSSION

Total polyphenol content

The free polyphenols (FPP) and bound polyphenols (BPP) fractions were analysed separately, and the calculated sum was reported as total polyphenol content (TPC). Both chocolate (Ch) and chocolate-coated popcorn (CCP) had significantly (P<0.05) greater amounts of FPP than plain popcorn (PP) alone. However, FPP content in Ch (802.89 ± 23.98 mg GAE/100g DW) was significantly (P<0.05) higher than those in CCP (639.74 ± 15.05 mg GAE/100g DW) and PP (87.01 ± 1.35 mg GAE/100g DW) **(Table 1)**. The same trend was observed for BPP and TPC fractions. Total polyphenol content (TPC) in all three samples varied significantly (P<0.05) from 1560.49 ± 21.84 mg GAE/100g DW to 1103.32± 13.58 mg GAE/100g DW and 505.25 ± 10.05 mg GAE/100g DW in Ch, CCP and PP, respectively **(Table 1)**. It was clear that TPC in plain popcorn was attributed mainly to BPP (418.24 ± 10.87 mg GAE/100g DW) in comparison with FPP (87.01 ± 1.35 mg GAE/100g DW). On the contrary, both CCP and Ch had significantly (P<0.05) higher FPP than BPP content. The FPP content detected in CCP and Ch were 639.74 ± 15.05 mg GAE/100g DW and 802.89 ± 23.98 mg GAE/100g DW, respectively **(Table 1 and Figure 1)**.

Table 1. Polyphenol content (mg GAE/100g DW. sample) and Trolox equivalent antioxidant capacity (TEAC) (mg TE/100g DW. sample) of different polyphenol fractions of popcorn (PP), chocolate-coated popcorn (CCP) and chocolate (C).

	PP	CCP	C
Free Polyphenols	87.01 ± 1.35^c	639.74 ± 15.05^b	802.89 ± 23.98^a
TEAC			
DPPH	65.70 ± 3.17^c	1102.00 ± 31.10^b	1155.35 ± 8.12^a
ABTS	77.29 ± 2.24^c	931.00 ± 53.2^b	1094.40 ± 55.0^a
Bound Polyphenols	418.24 ± 10.87^c	463.58 ± 8.49^b	757.60 ± 27.70^a
TEAC			
DPPH	321.23 ± 10.45^c	930.00 ± 43.40^b	1404.20 ± 69.60^a
ABTS	319.08 ± 14.46^c	872.40 ± 52.50^b	1284.10 ± 70.4^a
Total Polyphenols Contents	505.25 ± 10.05^c	1103.32 ± 13.58^b	1560.49 ± 21.84^a
TEAC			
DPPH	386.93 ± 11.45^c	2032.00 ± 40.0^b	2559.50 ± 69.1^a
ABTS	396.37 ± 15.4^c	1803.40 ± 65.3^b	2378.50 ± 108.0^a

Results expressed as means (n = 6) ± SD. Means within each row followed by different superscript letters are significantly different (P<0.05)

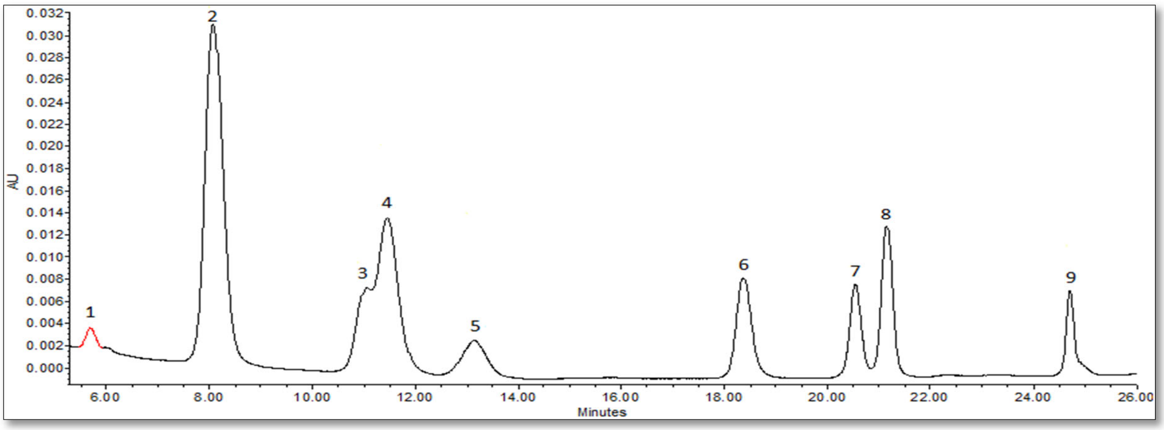

Figure 1. Chromatogram of polyphenol standards recorded at 290nm: 1 = gallic acid, 2 = (±)-catechin hydrate, 3 = vanillic acid, 4 = caffeic acid, 5 = (-)-epicatechin, 6 = *p*-Coumaric acid, 7 = *trans*-ferulic acid, 8 = sinapic acid and 9 = quercetin hydrate.

The amounts of FPP (87.01 ± 1.35 mg GAE/100g DW) and TPC (505 ± 10.05 mg GAE/100g DW) in PP were greater than those values reported in the literature for yellow popcorn popped without oil. A FPP content of 7 mg GAE/100g DW while TPC of 131 mg GAE/100g DW. Furthermore, the detected FPP content in PP was higher than in popcorn kernel (37 mg GAE/100g DW). On the other hand, TPC in PP reported in this study was lower than those in popcorn kernels of 539 mg GAE/100g DW and 797 mg GAE /100g DW, respectively. Such variations could be attributed to many factors, including, popcorn variety and method of preparation, extraction and analysis.

The BPP represent the main polyphenols in whole grains, and the calculated value from results in **Table 1** showed that BPP represented approximately 83% of TPC in plain popcorn. This percentage BPP content was within the range obtained for corn kernels (62-85%) [27,7], but lower than the 95% for popcorn kernels. Unfortunately, the literature on BPP Pin popped popcorn is lacking, and most studies only focused on FPP.

The FPP content measured in baking chocolate (803 mg GAE/100g DW equivalent to 800 mg GAE/100g FW) was within the values in dark chocolate(800 - 840 mg GAE/100g FW) [28], but smaller than those, 1816 mg GAE/100g FW [29]. The TPC (1560 mg GAE/100g DW equivalent to 1555 mg GAE/100g FW) **(Table 1)** was within the range (1176 to 2970 mg GAE/100g FW) [30], but smaller than the 5146 mg GAE/100g FW [31].

It has been estimated that in baking chocolate, about half of the polyphenols existed as BPP, mostly as condensed tannins [32,34]. Similarly, polyphenol content in dark chocolate consisted of 38% FPPs and 62% BPP; while in milk chocolate, FPP made up 53% and BPP made up 47% of total polyphenols [35]. It should be noted that polyphenol composition (FPP and BPP) detected in this current investigation did not agree with the findings of dark chocolate, but was similar to that of milk chocolate. This could possiblydue to the fact that measured BPP content using a different method and expressed the results as condensed tannin equivalents. Additionally, the presence of milk influenced polyphenol content. Milk solids are one of the raw materials used in the production of our baking chocolate sample, which could possibly cause the composition to be similar to that of milk chocolate. Another contributing factor to variations in reported result could be the methods of polyphenols extraction. Previous studies investigated the phenolic content in yellow popcorn and baking chocolate using aqueous organic solutions of methanol, ethanol and/or acetone [28,22]. These extraction conditions can only liberate the FPP, while the BPP remains in the residue [36,37]. consequently, the values reported by these authors underestimated TPC and were smaller than the amount reported in the current study.

Antioxidant Capacity

Trolox equivalent antioxidant capacity (TEAC) for free polyphenols (FPP) and bound polyphenols (BPP) were performed separately, using DPPH and ABTS assays. The TEAC in total polyphenols (TPP) were then calculated from the individual data of FPP and BPP fractions. Both DPPH and ABTS assays showed similar results, in which the FPP fraction in Ch and CCP had significantly ($P<0.05$) higher antioxidant capacity than in PP. Additionally, TEAC of FPP in Ch was significantly higher ($P<0.05$) than those in CCP. The same trend was observed for BPP. Similar to the previous findings of polyphenol contents, the TEAC in Ch was significantly ($P<0.05$) the highest, followed by those in CCP, and in PP in descending order for both FPP and BPP fractions **(Table 1)**.

The TEAC detected in the TPP fraction of PP [386.93 ± 11.45 (DPPH method) and 396. 37 ± 15.4 mg TE/100g DW (ABTS)] **(Table 1)** were within the range (128 – 96825 mg TE/100g DW) in popcorn kernels, and slightly smaller than those (400 - 501 mg TE/100g DW). The high TEAC in PP can be attributed to the significantly (P<0.05) large quantities of BPP (83%) compared with FPP (17%).

The TEACs of FPP measured in chocolate and on fresh weight bases for easy comparison with the literature values (1091 and 1151 mg TE/100g FW, using ABTS and DPPH, respictevely) were higher than the range in dark chocolate (881-961 mg TE/100g FW), and lower than (1972 to 3751 mg TE/100g FW). Additionally, the TEAC in the TPC fraction of chocolate (2370-2551 mg TE/100g FW) varied from literature values reported in dark and baking chocolates. The TEAC in Ch was greater than the amounts reported (3.78 – 621.22 and 2000 mg TE/100g FW, respectively), and smaller than those observed (3475-7357 TE/100g FW) [38,39].

The antioxidant capacities of FPPs and BPPs in Ch were not in accordance with their respective polyphenol contents. The FPP content (802.89 mg GAE/100 g DW) was larger than that in BPP (757.60 mg GAE/100 g DW). While, the TEAC in the BPP fraction (1404.2 mg TE/100g DW) was greater than in FPP (1155.35 mg TE/100 g DW) of chocolate. This inconsistency could be attributed to the presence of glycoside-bound polyphenols in BPP extracts, which have highly reactive scavenging compounds, causing BPP to have a higher antioxidant capacity than FPP [40-43].

On the contrary to the resuslts of TAEC in Ch, data in **Table 1** revelaed good corellation between the reported amounts of free and bound polyphenols and the TEAC in CCP. The larger contente of FPP (639.74 mg GAE/ 100 g Dw) yielded greater amount of TEAC (1102,00 mg TE/ 100 g DW). These resulsts are in agrement with the pervious suggestion that glycoside-bound polyphenols in BPP extracts contributed more to the antioxidant capacity. Consequently, the significantly the smaller amount of BPP (463.58 mg GAE/ 100 g Dw) in comparison with FPP (639.74 mg GAE/ 100 g DW) generated less TEAC in CCP.

The differences in measured antioxidant capacity using DPPH and ABTS methods could be due to the differences in reactivity of antioxidants in the analysed samples with the radicals in ABTS and DPPH [44].

Phenolic compounds in free polyphenol fractions

Using a mobile phase of methanol and acetic acid, two flavanol compounds (catechin, epicatechin), one flavonol (quercetin) and six phenolic acids (gallic acid, *p*-coumaric acid, ferulic acid, sinapic acid, vanillic acid, caffeic acid) were identified in the three samples (PP, Ch, & CCP). A total of nine polyphenol standards were applied **(Figure 1)**. The wavelength used to detect (±)-catechin hydrate, (-)-epicatechin and sinapic acid was 280 nm, while 290 nm was applied with gallic acid, vanillic acid, caffeic acid, *p*-coumaric acid, *trans*-ferulic acid and quercetin hydrate. The chromatograms obtained for free polyphenol fractions (FPP) in PP, CCP and Ch are shown in **Figure 2**. Epicatechin, vanillic acid and sinapic acid were not detected in PP **(Figure 2)**. However, Ch had significantly (P<0.05) greater amounts of epicatechin, vanillic acid, sinapic acid, gallic acid, catechin, caffeic acid and quercetin than CCP. Similarly, CCP showed the similar pattern with significantly (P<0.05) greater amounts of gallic acid, catechin, caffeic acid and quercetin than PP. *p*-coumaric acid concentrations in CCP (13.37 µg/g DW) and Ch (12.93 µg/g DWwere similar (P>0.05), and at the same time significantly (P<0.05) greater than that in PP (8.22 µg/g DW. Ferulic acid was the only polyphenol in PP with significantly (P<0.05) greater concentration compared with CCP and Ch **(Table 2)**.

Table 2. Concentrations of polyphenols (µg/g sample dry weight) * in plain popcorn (PP), chocolate-coated popcorn (CCP) and chocolate (Ch).

	PP	CCP	Ch
Gallic Acid	90.33 ± 1.81^{c}	131.88 ± 3.70^{b}	147.32 ± 7.79^{a}
Catechin hydrate	56.78 ± 3.20^{c}	500.17 ± 10.36^{b}	636.52 ± 10.98^{a}
Vanillic Acid	Not detected	30.04 ± 1.20^{b}	36.87 ± 1.39^{a}
Caffeic Acid	7.19 ± 0.42^{c}	34.37 ± 1.33^{b}	42.81 ± 1.76^{a}
Epicatechin	Not detected	356.38 ± 5.47^{b}	452.63 ± 5.00^{a}
***p*-Coumaric acid**	8.22 ± 0.088^{b}	13.37 ± 0.24^{a}	12.93 ± 0.49^{a}
Ferulic Acid	6.63 ± 0.37^{a}	4.80 ± 0.30^{b}	4.33 ± 0.24^{c}
Sinapic Acid	Not detected	9.51 ± 0.50^{b}	17.25 ± 1.00^{a}
Quercetin Hydrate	13.58 ± 0.78^{c}	75.39 ± 3.93^{b}	93.27 ± 2.11^{a}

Results are means (n=6) ± SD. Means within each row followed by different superscript letters are significantly different (P<0.05)
**Concentration of gallic acid was expressed in mg/g sample dry weight*

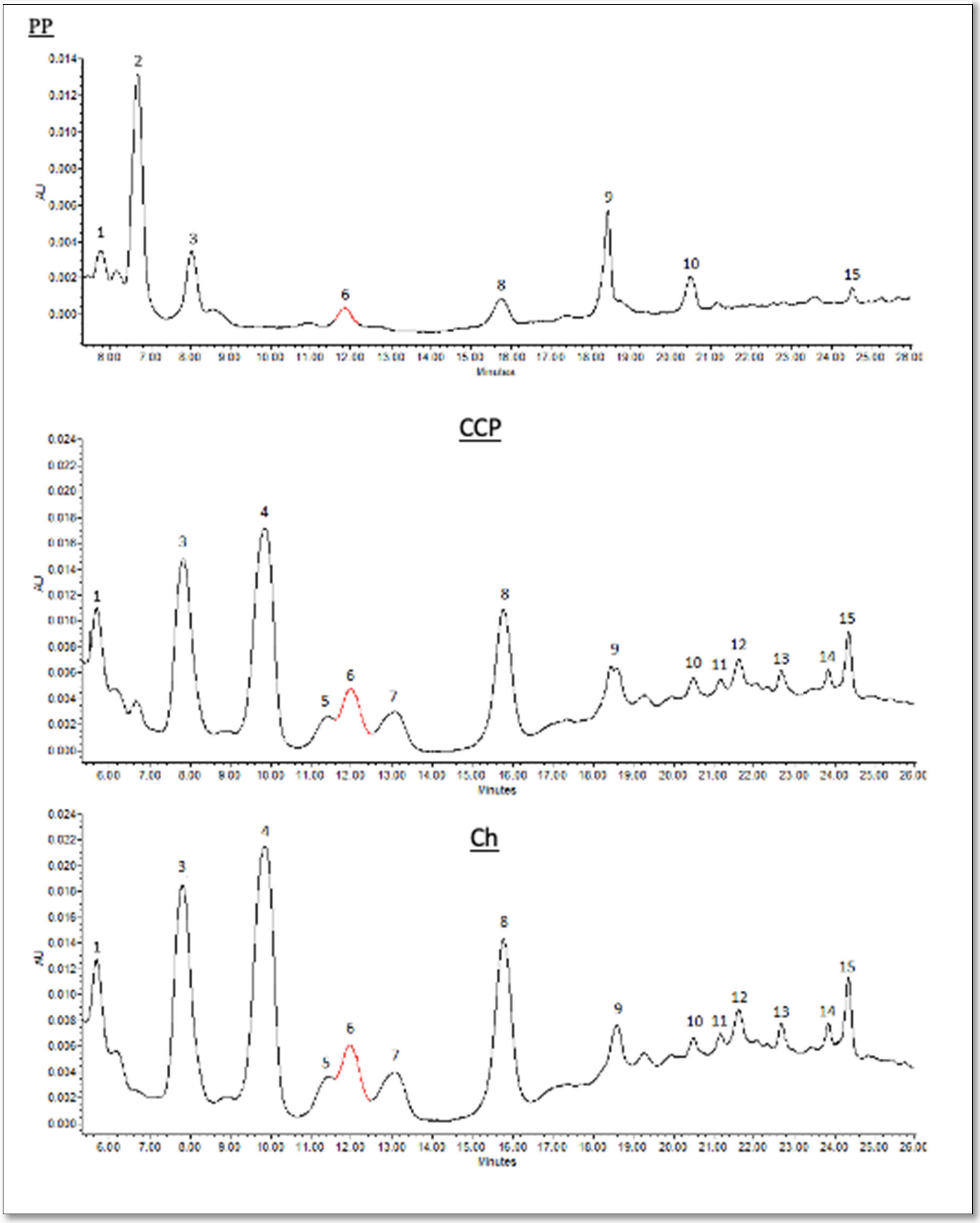

Figure 2. Chromatograms of various phenolic compounds detected at 290 nm in the FPP fractions of PP, CCP and Ch: 1 = gallic acid, 3 = (±)-catechin hydrate, 5 = vanillic acid, 6 = caffeic acid, 7 = (-)-epicatechin, 9 = *p*-coumaric acid, 10 = *trans*-ferulic acid, 11 = sinapic acid, 15 = quercetin hydrate

Gallic acid was the predominant polyphenol in all three samples (PP, CCP and Ch). More than 99% of FPP comprised of gallic acid. Consequently, gallic acid was expressed in mg/g sample DW, instead of µg/g sample DW, which was used with all other eight polyphenols. Following gallic acid, catechin was the second highest polyphenol in all three samples **(Table 2)**. Gallic acid concentration in Ch (147.32 mg/g DW) was much higher than the concentration reported by Tokusoglu and Ünal (2002) in dark chocolate (0.57-0.72 mg/g FW).

Catechin concentration measured in chocolate (0.64 mg/g FW, equivalent to 636.52 µg/g DW) was within the values for baking chocolate (0.112-0.727 mg/g FW for chocolate (0.11-1.17 mg/g FW); and for sweetened chocolate (0.63-0.66 mg/g FW) [45]. On the other hand, catechin concentration was higher than in baking chocolate (0.24 mg/g FW), and lower than (1.848 mg/g FW) [33].

The detected amounts of vanillic acid in CCP and Ch in this current study (30.04 and 36.87 µg/g DW, respectively) were significantly greater ($P<0.05$) thanin chocolate milk (0.019 mg/g FW). However, both vanillic and sinapic acids were not detected in PP in this study.

Caffeic acid concentration in PP (7.19 µg/g DW) was much higher than those reported (0.013 µg/g DW). However, caffeic acid concentration was within the values reported in popcorn kernels 0.014 -9.83 µg/g DW. Caffeic acid concentration (0.0428 mg/g DW, equivalent to (7.19 µg/g DW) was much lower than the concentration reported in baking chocolate (0.32 mg/g FW). These discrepancies could be due to differences in the methods of analysis and extraction solvents, as different solvents will affect the recovery of polyphenols [46].

Epicatechin concentration in Ch (0.451 mg/g FW, equivalent to 0.453 mg/g DW) was within the range for baking chocolate (0.412-1.223 mg/g FW), but lower than the range (0.52 mg/g FW). Epicatechin is widely acknowledged as the main flavanol compound in chocolate. However, catechin concentration in Ch was higher than epicatechin [38,47] **(Table 2**).

P-coumaric acid concentration in PP (8.22 µg/g DW) was greater than the value reported in popcorn (0.005 µg/g DW), and smaller than the value in popcorn kernels (16.31 µg/g DW). Likewise, ferulic acid concentration in PP (6.63 µg/g DW) was greater than the amount (0.006 µg/g DW) in popcorn, and close to that in popcorn kernel (5.94 µg/g DW) [48].

Quercetin was detected in all tested samples (PP, CCP and Ch) with concentrations ranging from 93.27 µg/g DW in Ch to 13.58 µg/g DW in PP. The detected amount of quercerin in PP was much greater than the concentration in popcorn kernels (7.6 µg/g DW). It should mention also that neither detected quercetin in popcorn [49-52].

Due to limited standards availability at the time of this investigation, peaks 2 and 8 in PP and peaks 4 and 8 in CCP and Ch **(Figure 2)** were not identified.

CONCLUSION

The present study showed that covering popcorn with chocolate produced a snack with more polyphenol content and antioxidant capacity from chocolate and more fiber from popcorn. Consequently, the CCP can be considered a healthier snack than the indifidual PP and Ch [53-56].

Further investigation into other antioxidants such as carotenoids from popcorn, which is known to be rich in carotenods, is suggested. Additionally, bioaccessibility / bioavailability tests are recommended to determine the nutritional value of CCP compared with PP and Ch.

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