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Effect of cultivar and ripening on the polyphenol contents of East African highland bananas (*Musa* spp.)

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Abstract

East African highland bananas (EAHBs) contain high amount of phenolics especially tannins, and are used to produce low-viscosity banana juice by a purely mechanical process. Occasional juice failure and cloudy appearance are the major problems facing juice production. The present work thus examined the variations in phenolic content of EAHB cultivars and their changes during ripening. The aim was to obtain a better knowledge of the various forms and amounts of phenolic compounds in different EAHBs, and how these properties may affect the ability of cultivars to produce low viscosity banana juice. Eleven banana cultivars including juice-producing and cooking bananas were harvested at the green maturity stage and analysed for total phenolic content (TPC), tannin content (TC), and tannin monomers at different ripening stages for five days. Analyses of TPC and TC were performed using the Folin-Ciocalteu method, whereas tannin monomers were identified by High-Performance Liquid Chromatography (HPLC) with UV detection. Multivariate analysis of variance was used to evaluate the relationship between cultivar, ripeness stage, and TPC/TC. A substantial difference in TPC was observed between juice-producing and cooking cultivars. The highest TPC was found in the juice-producing cultivar Kibungara (360.68 ± 17.12 mg GAE/100 g) at day 5 (the ripe stage), while the lowest TPC (8.67 ± 0.22 mg GAE/100 g) was observed in the cooking cultivar Malindi at ripening day 5. The results revealed that TPC and TC of banana pulp seemed to be more related to cultivar ($p \leq 0.05$) than physiological changes during ripening ($p \geq 0.05$). Further, HPLC analysis showed that among the individual catechins, gallocatechin was the predominant monomer in juice-producing cultivars, whereas in cooking cultivars, gallic acid was dominant. The present work indicated that high amount of total phenolic such as tannins especially gallocatechin in juice-producing banana cultivars favour the release of banana juice, and that analysis of phenolic compounds will provide a basis in the selection of banana cultivars with high potential for juice production.

Introduction

East African highland bananas (EAHB; *Musa* spp.) are common banana species in East Africa, and mostly utilised for juice production and cooking (Davies, 1995). Juice-producing bananas can be differentiated from cooking bananas by their ability to release juice through mechanical processing (Kyamuhangire and Pehrson, 1997). Other unique characters of a juice-producing banana as compared to cooking banana are the high tannin content and strong astringent taste (Kyamuhangire *et al.,* 2006). The high content of tannin in EAHB has been linked to their ability to produce a clear and low-viscosity banana juice (Kyamuhangire *et al.,* 2006; Kibazohi *et al.,* 2017).

To obtain low-viscosity banana juice, the EAHB are mechanically processed by blending a mixture of ripe bananas for extended time until juice is extracted (Kibazohi *et al.,* 2017; Majaliwa *et al.,* 2019). Among the challenges associated with mechanical banana juice processing is juice extraction failure which has mostly been observed for overripe bananas (Kyamuhangire and Pehrson, 1999). Kibazohi *et al.* (2017) observed a decrease in the content of condensed tannins with the ripening of juice-producing banana followed by juice extraction failure. It is hypothesised that condensed tannins are involved in the formation of insoluble tannin-protein complexes that facilitates juice extraction. However, information about which phenolic compounds that are involved in juice release is limited and more research is needed to obtain more efficient juice processing.

The phenolic composition of edible fruits

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varies depending on cultivar, stage of ripening, maturity, and location (Harris, 1975; Mozafar, 1994; Lee and Kader, 2000). Ripening of fruits involves a series of complex biochemical reactions that alter the phytochemical composition of fruits (Speirs and Brady, 1991). A decrease in phenolic compounds with fruit ripening has been reported in mango (Abu-Goukh and Abu-Sarra, 1993), date (Al-Ogaidi and Mutlak, 1986), and banana (Ibrahim *et al.,* 1994). However, a decrease in astringency during ripening has been linked with an increased polymerisation of leucoanthocyanidins (Goldstein and Swain, 1963). On the contrary, some fruits have shown an increase in phenolic content with ripening probably due to the increase of biosynthesis of anthocyanins (Mazza, 2018).

Despite the great commercial potential of EAHBs for juice processing, there have only been few studies focusing on phenolic content in them (Kyamuhangire *et al.,* 2006; Kibazohi *et al.,* 2017), but no comparison between different EAHB cultivars and how they are influenced by cultivar and ripening.

The aim of the present work was to compare juice-producing and cooking (non-juice-producing) banana cultivars by characterisation and quantification of individual and total phenolic compounds in 11 banana cultivars, and to gain an understanding of the mechanisms behind juice release during banana juice processing.

Materials and methods

Seven EAHB banana cultivars namely Yangambi KM 5, Nshanshambile, Mtwishe, Nyengele, Nshakala, Malindi, and FIA 23 were sampled from the household farms in Muleba district, Kagera region, Tanzania. The banana samples of Shimbira, Ndeshi Laini, Nyengele, and Ng'ombe Mronge cultivars were collected from the household farms in Rombo district, Kilimanjaro region, Tanzania. Muleba district (1°45' N, 31°49' E) has an average temperature of 20°C, and an average rainfall of 1100 mm per annum (Theodory, 2017). Rombo district (3°10' N, 37°33' E, 3°09' S) has an average temperature of 14 - 20°C, and an average rainfall of 1200 - 2000 mm per annum (Mbwiga, 2016; Fundisha, 2020).

Fruit sampling

Eleven banana genotypes from different household farms were selected and collected in May 2018 at peak maturation. Bananas were harvested at the matured green stage (based on fruit fullness), and from each bunch, banana fingers were plucked from the top, middle, and bottom position of bunches to obtain representative samples. The bananas were then ripened in the laboratory at 28 - 32°C atmospheric temperature with 95 - 100% relative humidity. After ripening, the fruits were washed and peeled. The peeled bananas were cut into slices (approximately 5 mm thick), put into liquid nitrogen, and then stored in a cryogenic freezer at -80°C until further analyses. All samples were analysed in triplicates.

Chemicals and reagents

Folin-Ciocalteau phenol reagent, anhydrous sodium carbonate (Na_2CO_3), and polyvinyl-polypyrrolidone ($(C_6H_9NO)_n$, PVPP) were purcahsed from Fluka®Analytical (Germany). Methanol (CH_3OH), trifluoroacetic acid (TFA) ($C_2HF_3O_2$), acetic acid (CH_3COOH), phenolic standards catechin ($C_{15}H_{14}O_6$), epicatechin ($C_{15}H_{14}O_6$), epigallocatechin ($C_{15}H_{14}O_7$), gallocatechin ($C_{15}H_{14}O_7$), gallic acid ($C_7H_6O_5$), quercetin ($C_{15}H_{10}O_7$), and epigallocatechin gallate ($C_{22}H_{18}O_{11}$) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Preparation of samples for total phenolic content (TPC) and tannin content (TC) analysis

Samples for TPC and TC were prepared according to Howard *et al.* (2003). Freeze-dried samples were ground to powder, and about 0.2 g of each powdered sample was weighed in a test tube. The extraction of phenolic compounds was performed by adding 5 mL of acidified methanol (MeOH:H_2O [70:30 v/v] plus 1% TFA) into the test tube. The mixture was then vortexed for 15 s, sonicated using an ultrasonic cleaner (Bransonic 8510, USA) for 10 min, and incubated in a water bath for 30 min at 60°C while shaking at 100 rpm. Subsequently, the mixture was put on ice to cool, sonicated again for 15 s, and centrifuged (Multifuge I.S.R Heraeus, Kandro Laboratory, Germany) at 5,000 *g* at 4°C for 5 min, followed by collection of the supernatant as an extract. The sediments were re-extracted again with 5 mL extraction solution, and the above steps were repeated to collect the second supernatant. The first and second extracts were mixed and stored at 20°C until analysis. Prior to analysis, extracts were centrifuged (IEC Micromax, USA) at 15,000 rpm for 5 min.

Preparation of standard for HPLC analysis

A standard stock solution was prepared by dissolving 1 mg of the standards in 1 mL of methanol. Stock solutions were combined into one single solution according to the expected relative proportions of each compound in the targeted banana pulp. The single

solution was further diluted in order to prepare for different working solutions. Subsequently, the standards were filtered with a 0.22 µm filter syringe (Whatman® GD/X) before injection into the HPLC system. Calibration curves were fitted for each standard using six different concentrations corresponding to the appropriate range for each compound (10 - 60 mg L^{-1}).

Preparation of samples for HPLC analysis

Banana pulp powder was defatted using the Soxhlet method (Stalikas, 2007; Tesfaye and Tefera, 2017) to remove any interfering lipids before the HPLC analysis. About 40 g banana pulp powder was inserted into thimble, and covered at the top with cotton wool, then 200 mL of 95% *n*-hexane was poured in the distillation flask. The Soxhlet instrument was assembled by putting distillation flasks, extraction chambers with siphon arm, and condensers together on a heating mantle. The heating mantle was then switched on while allowing cooling water to flow through the condensers. Subsequently, the chamber containing the sample was filled with a warm solvent. When the solvent almost full, the siphon will remove the solvent together with dissolved compounds in it back to the distillation flask. This procedure was repeated until the extraction was complete. Thereafter, the mixture was allowed to cool and then dried in an oven at 60°C for 30 min to remove the solvent.

Determination of total phenolic content (TPC) and tannin content (TC)

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method previously described by Makkar (2003). The tannin content (TC) was determined by combining the Folin-Ciocalteu method with the addition of PVPP. TPC and TC concentrations were expressed as mg gallic equivalents (GAE) per 100 g of dry weight. Briefly, 1 mL of the extract was diluted ten times, and then mixed with 0.25 mL of Folin-Ciocalteu reagent (diluted 1:4 with distilled water). The mixture was left to stand at room temperature for 8 min before adding 0.25 mL of saturated sodium carbonate solution. The mixture was vortexed, and allowed to stand at room temperature for 10 min, and then centrifuged at 15,000 rpm (IEC Micromax, USA) for 5 min before reading the absorbance at 765 nm using a UV absorbance spectrophotometer (Safire II Plate Reader, Tecan, Austria).

TC was determined by adding 1 g of PVPP powder to 1 mL of the mixture used above, and the second absorbance measurement was made again at 765 nm. The difference between the first and second absorbance values was used to calculate the tannin concentration.

Determination of tannin monomers

The tannin monomers, namely catechin (C), epicatechin (EP), gallocatechin (GCT), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and the phenolic plant metabolites namely gallic acid (GA) and quercetin (QC) in the banana pulp were determined following the method of Dhanani *et al.* (2017) with slight modifications to suit a banana matrix. HPLC 1260 Infinity II Agilent Technologies System (Germany) comprised of a UV detector operating at 280 nm was employed for quantification of tannin monomers. Separation was achieved with a Zorbax C_{18} column (250 × 4.6 mm, 5 µm) set at 30°C. Gradient elution was applied with water and acetic acid (98/2, v/v/v) as solvent A, and a mixture of methanol and acetic acid (98/2, v/v/v) as solvent B. The gradient was run with an injection volume of 10 µL, and a flow rate of 1.0 mL min^{-1}. The tannin monomers were identified by comparison of the retention times of HPLC peaks of the standards and samples.

Method validation

For the routine application of the developed method for analysis of banana pulp, the method was validated for linearity, LOD, LOQ, accuracy, and precision.

Statistical analysis

The results were expressed as mean values ± standard deviations from at least three replicates. A multivariate analysis of variance (MANOVA) using SPSS statistics version 24 was used to test the main effects and interaction of independent variables on dependent variables.

Results and discussion

Effect of cultivar on total phenolic content (TPC) and tannin content (TC)

The results showed that the variation in TPC and TC contents with cultivar was statistically significant ($p \leq 0.05$) with the Eta-squared (η^2) of 0.789 and 0.850 respectively, as indicated by MANOVA analysis (Table 1). The juice-producing cultivars, Kibungara (*Pisang Awak*) and Yangambi KM 5 had the highest TPC with the mean values of 360.68 ± 17.12 and 332.76 ± 6.47 mg GAE 100 g DW^{-1}, respectively. The cooking cultivar, Malindi cultivar (Dwarf Cavendish) had the lowest TPC (8 ± 0.22 mg GAE 100 g DW^{-1}) as shown in Table 2. In previous studies with other cultivars, lower TPC

Table 1. Multivariate analysis of the effects of cultivar and ripening on total phenolic content (TPC) and tannin content (TC) in juice-producing and cooking banana cultivars.

Source	Dependent variable	df	Mean square	F	Sig.	Effect size
Banana cultivar (A)	Total phenolic content	11	0.941	8.523	0.000***	0.789
	Tannin content	11	1.098	12.871	0.000***	0.850
Ripening day (B)	Total phenolic content	1	0.018	0.162	0.690	0.006
	Tannin content	1	0.001	0.007	0.932	0.000
A*B	Total phenolic content	51	0.063	0.574	0.954	0.539
	Tannin content	51	0.060	0.703	0.858	0.589
Error	Total phenolic content	25	0.110			
	Tannin content	25	0.085			

Effect size = partial η2; and *** = $p \leq 0.001$.

values was reported as compared to the observed values in the present work. *Pisang Mas* and *Pisang Awak* bananas have been reported to have a phenolic content between and 51 - 56 and 90.4 mg GAE 100 g DW^{-1}, respectively (Sun *et al.,* 2002; Lim *et al.,* 2007; Alothman *et al.,* 2009; Choo and Azis, 2010). Septembre-Malaterre *et al.* (2016) reported TPC values ranging from 1.48 to 1.74 mg GAE 100 g DW^{-1} for Cavendish bananas (*Musa acuminata*) from Réunion French Island. The variations in TPC and TC across cultivars are probably related to differences in agro-ecological conditions (Fatemeh *et al.,* 2012), genetic factors, and environmental stresses.

The distribution of TC among banana cultivars exhibited a pattern similar to the one observed for TPC, with some differences. As shown in Table 2, juice-producing cultivars contained higher amounts of tannins than the cooking cultivars. Kibungara cultivar had the highest mean (331.25 ± 0.01 mg GAE 100 g DW^{-1}), followed by Yangambi KM 5 (256.06 ± 0.15 mg GAE 100 g DW^{-1}), while the Malindi cultivar had the lowest tannin content (3.41 ± 0.00 mg GAE 100 g DW^{-1}). This may explain the common use of Kibungara and Yangambi KM 5 for banana juice extraction (Kyamuhangire and Pehrson, 1999; Kyamuhangire *et al.,* 2006; Kibazohi *et al.,* 2017). This is also in line with the study by Kyamuhangire *et al.* (2006) which reported a higher content of tannins in juice-producing cultivars than in cooking cultivars (Matoke). The present work showed that despite substantially higher TPC (Table 2) in Ng'ombe Mronge cultivar, it did not result in juice release probably due to low TC, hence tannins (not just phenolics) are likely a key factor to juice release.

Effect of ripening on TPC and TC

The TPC of the juice-producing and cooking cultivars in banana samples from five different ripening days are shown in Table 2. Ripening did not induce any significant differences in terms of TPC and TC. The results showed that the phenolic content in juice-producing cultivars increased slightly with ripening except for Mtwishe, which showed a decreased level from day 1 to 5. Previous studies by Sulaiman and Ooi (2012), Newilah *et al.* (2010), and Giovanelli *et al.* (1999) have reported a significant increase in phenolic compounds during ripening,

In the present work, the TPC in cooking cultivars decreased during ripening except for Shimbira and FIA 23 cultivars, both showing increase from day 1 to 3, and then a decrease from day 4 to 5. A decrease in phenolic content with ripening has also been observed in fruits like guava and avocado (Bashir and Abu-Goukh 2003; Zhang *et al.,* 2013). The variation in phenolic content with ripening in most of the banana cultivars might be attributed to the physiological conditions, natural chemical compositions (Dixon and Paiva, 1995; Huang *et al.,* 2005), and different activities of enzymes responsible for the production of biochemical compounds (Parr and Bolwell, 2000; Raffo *et al.,* 2006). In general, the interactive effect of the banana cultivars and ripening days on both TPC and TC content was not significant ($p \geq 0.05$).

Identification of tannin monomers

In the present work, seven tannin monomers were identified in banana pulp extracts using standards (Figure 1). Among the identified monomeric flavan-3-ols in the banana pulp, gallocatechin was the main compound in juice-producing cultivar (Yangambi KM 5) with 390.12 ± 0.23 mg 100 g DW^{-1} (Table 3), which was 20-fold greater than the highest concentration (18.45 ± 0.73 mg 100 g DW^{-1}) found in the cooking cultivar (Malindi). Kibungara, Nyengele, and

Table 2. Total phenolic content (TPC; mg GAE 100 g DW^{-1}) and tannin content (TC; mg GAE 100 g DW^{-1}) in juice-producing and cooking banana cultivars.

Cultivar	Day 1		Day 2		Day 3		Day 4		Day 5	
	TPC	TC	TPC	TC	TPC	TC	TPC	TC	TPC	TC
Yangambi Km5**	214.54 ± 14.02	174.26 ± 0.32	218.10 ± 1.70	176.11 ± 0.21	229.18 ± 9.32	179.10 ± 0.14	255.15 ± 17.15	200.53 ± 0.13	332.76 ± 6.47	256.06 ± 0.15
Mtwishe**	214.21 ± 8.56	167.06 ± 0.22	216.04 ± 3.22	167.19 ± 0.13	200.29 ± 8.10	167.80 ± 0.10	198.10 ± 1.90	172.04 ± 0.14	191.44 ± 10.97	179.35 ± 0.02
Nshanshambile**	144.32 ± 10.78	116.23 ± 0.19	202.21 ± 3.76	155.30 ± 0.09	231.24 ± 10.11	193.01 ± 0.13	235.73 ± 6.42	165.21 ± 0.17	238.60 ± 9.56	223.14 ± 1.02
Kibungara**	335.05 ± 15.17	298.17 ± 0.98	337.36 ± 1.20	300.99 ± 1.20	340.65 ± 11.08	304.02 ± 1.43	342.51 ± 12.05	331.23 ± 0.95	360.68 ± 17.12	331.25 ± 0.01
Nyengele**	110.12 ± 6.43	67.08 ± 0.04	103.22 ± 1.04	67.02 ± 0.01	99.73 ± 5.38	69.08 ± 0.06	92.71 ± 4.23	74.28 ± 0.06	87.50 ± 1.15	80.03 ± 0.03
Shimbira**	90.34 ± 5.02	63.05 ± 0.01	100.06 ± 1.10	63.52 ± 0.05	101.37 ± 4.22	64.77 ± 0.01	93.40 ± 1.65	54.61 ± 0.03	96.23 ± 2.12	44.00 ± 0.01
FIA 23*	64.42. ± 1.89	35.28 ± 0.01	71.03 ± 1.87	35.26 ± 0.06	79.33 ± 4.52	35.24 ± 0.04	65.01 ± 3.55	36.22 ± 0.02	68.20 ± 0.09	38.11 ± 0.01
Nshakala*	82.73 ± 3.12	30.11 ± 0.02	80.13 ± 0.28	30.25 ± 0.04	78.50 ± 0.67	30.02 ± 0.03	74.21 ± 1.89	31.90 ± 0.04	72.06 ± 1.15	32.15 ± 0.02
Malindi*	5.36 ± 0.36	4.98 ± 0.00	5.09 ± 0.06	4.15 ± 0.00	6.67 ± 0.12	4.22 ± 0.00	7.70 ± 0.09	3.07 ± 0.00	8.67 ± 0.22	3.41 ± 0.00
Ndeshi Laini*	82.29 ± 3.12	53.02 ± 0.02	69.09 ± 0.28	55.06 ± 0.01	63.33 ± 1.50	56.64 ± 0.08	43.12 ± 2.34	56.40 ± 0.03	33.63 ± 0.35	60.13 ± 0.04
Ng'ombe Mronge*	247.58 ± 11.08	15.10 ± 0.03	194.14 ± 1.34	24.45 ± 0.01	176.06 ± 12.04	34.13 ± 0.03	158.09 ± 4.12	66.02 ± 0.02	124.45 ± 5.61	102.11 ± 0.50

DW = dry weight; * = cooking cultivars, and ** = juice-producing cultivars. Values are mean ± standard deviation.

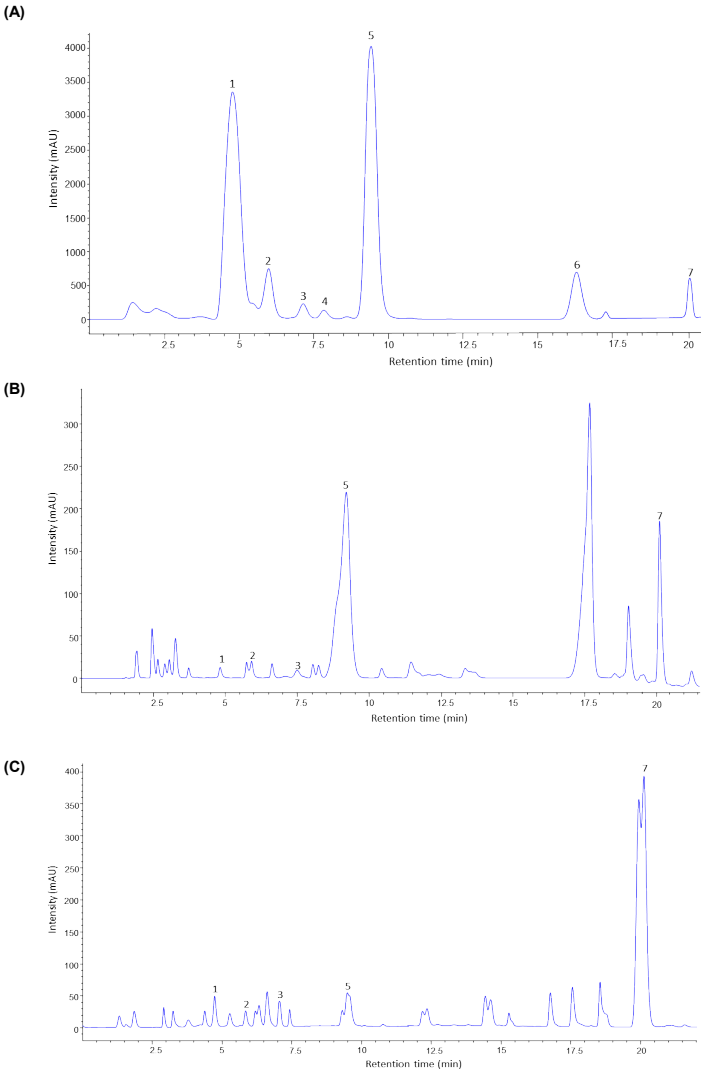

Figure 1. HPLC chromatogram of tannin monomers in the two banana cultivars: (A) standard, (B) juice-producing cultivar (Kibungara), and (C) cooking cultivar (FIA 23). The peaks were identified as: (1) gallic acid, (2) quercetin, (3) catechin, (4) epicatechin, (5) gallocatechin, (6) epigallocatechin, and (7) epigallocatechin gallate.

Nshanshambile, which are well known among farmers as good raw materials for low viscosity banana juice, contained more than 45 mg 100 g DW^{-1} of gallocatechin. The higher gallocatechin content of the juice cultivars are likely related to their higher amount of condensed tannins. Similar results were observed by Someya *et al.* (2002) who reported high amounts of gallocatechin in banana pulps of *Musa* spp. cultivars. It is also evident from the literature that various soluble phenolic compounds such as gallic acid, catechin, gallocatechin, naringenin, and epigallocatechin 3-*O*-gallate exist in the pulp of banana fruits (Méndez *et al.,* 2003; Aurore *et al.,* 2009; Septembre-Malaterre *et al.,* 2016). Further, gallic acid was present in all the cultivars, and on average, the cooking cultivars had higher contents of gallic acid (5.20 ± 0.90 mg 100 g DW^{-1}) than the juice-producing cultivars (2.01 ± 0.24 mg 100 g DW^{-1}).

Catechin was present in all juice-producing and cooking cultivars with the exception of Malindi and Nshakala cultivars. Kibungara had the highest amount of catechin (23.04 ± 0.51 mg 100 g DW^{-1}) followed by Nshanshambile (12.11 ± 0.89 mg 100 g DW^{-1}). This observation agrees with the findings of Bennett *et al.* (2010) and Méndez *et al.* (2003). Significant levels of catechins were also detected in line with the observation of Harnly *et al.* (2006).

Epicatechin was not identified in any of the cultivars which agrees with other studies reporting negligible amounts of epicatechin in the range of 0.0179 - 0.1984 mg g^{-1} in ripe and unripe banana fruits, respectively (Bennett *et al.,* 2010). The epigallocatechin gallate content varied, and was not found to be linked to juice-producing or cooking cultivar. FIA 23 had the highest content (14.72 ± 2.03 mg 100 g DW^{-1}) followed by KM 5 (7.35 ± 0.07 mg 100 g DW^{-1}) and Kibungara (6.53 ± 0.75 mg

Table 3. Tannin monomers identified in juice-producing and cooking banana cultivars.

Cultivar	Tannin monomer (mg 100 g DW^{-1})						
	GA	QE	CT	EPCT	GCT	EPGCT	EPGCTG
Ndeshi Laini*	2.45 ± 0.04	0.02 ± 0.00	3.77 ± 0.61	nd	nd	1.69 ± 0.11	0.98 ± 0.06
Shimbira*	2.62 ± 0.05	0.01 ± 0.00	1.48 ± 0.21	nd	nd	3.62 ± 0.17	1.03 ± 0.11
FIA 23*	3.29 ± 0.19	2.10 ± 0.05	0.93 ± 0.07	nd	12.18 ± 0.87	7.75 ± 1.22	14.72 ± 2.03
Nshakala*	3.35 ± 0.13	0.92 ± 0.12	nd	nd	nd	nd	0.07 ± 0.00
Nshanshambile**	1.45 ± 0.01	1.22 ± 0.03	12.11 ± 0.89	nd	45.38 ± 5.11	0.84 ± 0.05	2.01 ± 0.02
Mtwishe**	0.89 ± 0.06	0.46 ± 0.00	5.36 ± 0.09	nd	26.13 ± 1.19	11.27 ± 3.22	4.14 ± 0.21
Ng'ombe Mronge*	1.56 ± 0.21	1.34 ± 0.04	8.53 ± 0.45	nd	28.23 ± 1.67	nd	0.16 ± 0.01
Yangambi KM 5**	0.56 ± 0.03	0.88 ± 0.02	0.84 ± 0.02	nd	390.12 ± 0.23	8.55 ± 0.14	7.35 ± 0.07
Nyengele**	2.00 ± 0.41	0.33 ± 0.01	6.52 ± 0.33	nd	58.32 ± 2.32	nd	0.41 ± 0.00
Kibungara**	2.01 ± 0.24	0.34 ± 0.11	23.04 ± 0.51	nd	190.04 ± 3.14	4.68 ± 0.33	6.53 ± 0.75
Malindi*	5.20 ± 0.90	0.87 ± 0.00	3.77 ± 0.35	nd	18.45 ± 0.73	11.33 ± 0.38	5.67 ± 0.23

DW = dry weight; nd = not detected; * = cooking cultivar; ** = juice-producing cultivar; GA = gallic acid; QE = quercetin; CT = catechin; EPCT = epicatechin; GCT = gallocatechin; EPGCT = epigallocatechin; and EPGCTG = epigallocatechin gallate. Values are mean ± standard deviation.

Table 4. Validation parameters of the developed HPLC-UV method.

Compound	Linear range (mg L^{-1})	Equation	R^2	LOD (mg L^{-1})	LOQ (mg L^{-1})	% RSD	
						Intra day (n = 6)	Inter day (n = 5)
Gallic acid	10 - 60	Y = 10187x - 10381	0.9998	1.99	6.04	2.6	3.2
Gallocatechin	10 - 60	Y = 13187x + 39619	0.9999	1.54	4.67	0.7	0.9
Epigallocatechin	10 - 60	Y = 14600x + 627.87	0.9999	1.35	4.08	3.0	1.8
Epicatechin	10 - 60	Y = 8239x + 3333	0.9999	1.56	4.78	0.6	0.8
Quercetin	10 - 60	Y = 14407x + 4758	0.9998	2.12	6.44	1.4	2.2
Catechin	10 - 60	Y = 14368x + 10460	0.9998	2.72	8.26	1.5	1.8
Epigallocatechin gallate	10 - 60	Y = 10081x + 4333	0.9995	3.39	10.27	2.8	2.1

R^2 = square of regression coefficient; LOD = limit of detection; LOQ = limit of quantification; and RSD = relative standard deviation.

Table 5. Recovery of tannin monomers in juice-producing and cooking banana cultivars.

Concentration spike (mg L^{-1})	Type	Analyte (%)						
		GA	GCT	EGC	EP	QC	C	EGCG
5	Kibungara (#1)	100.2 ± 2.1	99.7 ± 2.1	98.5 ± 1.1	97.3 ± 1.6	105.2 ± 0.9	97.3 ± 2.5	101.5 ± 0.8
	Nyengele (#5)	102.6 ± 0.6	98.6 ± 2.2	100.3 ± 2.1	101.1 ± 2.7	100.2 ± 0.7	104.2 ± 0.8	100.9 ± 1.5
	Nshanshambile (#9)	102.3 ± 2.1	94.3 ± 1.8	101.6 ± 2.9	96.3 ± 1.9	99.4 ± 1.8	96.1 ± 1.5	102.3 ± 2.5
30	Kibungara (#1)	100.7 ± 1.6	96.8 ± 2.7	104.3 ± 1.5	95.8 ± 2.0	95.7 ± 2.3	99.1 ± 1.1	96.4 ± 2.7
	Nyengele (#5)	96.3 ± 0.3	97.9 ± 2.9	97.8 ± 0.2	98.5 ± 2.2	98.3 ± 1.8	95.8 ± 2.1	97.4 ± 1.2
	Nshanshambile (#9)	97.0 ± 0.8	99.2 ± 1.1	96.1 ± 1.6	97.6 ± 2.8	101.1 ± 0.6	96.7 ± 0.8	96.6 ± 1.9
50	Kibungara (#1)	99.1 ± 2.6	95.4 ± 0.7	103.4 ± 2.1	94.2 ± 1.1	102.3 ± 2.1	98.1 ± 1.2	98.8 ± 1.4
	Nyengele (#5)	103.5 ± 1.9	95.2 ± 1.9	98.7 ± 0.9	95.1 ± 2.0	103.2 ± 1.9	97.2 ± 2.3	100.2 ± 0.8
	Nshanshambile (#9)	102.9 ± 1.1	95.6 ± 1.9	102.9 ± 2.1	97.5 ± 1.1	96.3 ± 1.4	104.2 ± 1.3	99.9 ± 2.1

= sample number; GA = gallic acid; GCT = gallocatechin; ECG = epigallocatechin; EP = epicatechin; QC = quercetin; C = catechin; and EGCG = epigallocatechin gallate. Values are mean ± standard deviation.

100 g DW^{-1}). Similarly, epigallocatechin gallate values (8 - 15 mg 100 g^{-1}) have been found in banana samples from Phitsanulok, Thailand (Pramote *et al.,* 2018).

Method validation

Calibration curves were found to be linear over the concentration range (10 - 60 mg L^{-1}). All the analytes showed good linearity with the coefficient of determinations (R^2) ranging from 0.9995 to 0.9999 for the seven phenolic standards (Table 4). The reproducibility (RSD) on the same and over different days was carried out, and found to be less than 3.2% for all experiments. Recovery studies were carried out by spiking three concentrations of the mixture of standards to each type of banana sample (Table 5). Good recoveries of 94.2 - 105.4% were found for all the spiked samples, which revealed that the current method was reliable, accurate, and reproducible. However, since HPLC-UV is more confounded with co-elution and matrix effects, further studies will then be required to validate our results using LC-MS.

Conclusion

The results obtained in the present work showed that banana pulp contained a wide range of phenolic compounds. The composition of phenolic compounds in banana varied significantly ($p \leq 0.05$) with cultivar. Ripening did not induce any significant ($p \geq 0.05$) differences in TPC and TC. Markedly higher levels of TPC, TC, and gallocatechin were found in juice-producing cultivars as compared to cooking cultivars. The high amount of total phenolic compounds and tannins, especially gallocatechin, in juice-producing banana cultivars may favour the formation of protein-phenolic complexes, thus facilitating banana juice release. Further research is needed in terms of identification of other biochemical characteristics across EAHBs, as well as understanding how protein-phenolic interactions may affect banana juice release.

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