Phenolic compound profile of commercial and non-commercial unripe banana flour using LC-DAD-ESI-MS

Tonna A. Anyasi[a*], Afam I.O. Jideani[a], Godwin R.A. Mchau[b]

[a]Department of Food Science and Technology
[b]Department of Horticultural Sciences
School of Agriculture, University of Venda, Private Bag X5050 Thohoyandou 0950, Limpopo Province, South Africa

DST-NRF
Centre of Excellence
in Food Security

University of Venda

PRESENTATION OVERVIEW

- Introduction
- Justification
- Objective of study
- Materials and method
- Results and discussion
- Conclusion and recommendation
- Selected references

University of Venda

INTRODUCTION

- Present day choices on which particular food produce to consume is now increasingly determined by factors such as health available and beneficial nutrients inherent in these foods.
- *Musa* spp is a highly consumed fruit all over the world and contains nutrients such as dietary fibers, minerals, vitamins, pro-vitamins and phenolic compounds (Vilela et al., 2014; Facundo et al., 2015) in varying concentrations.
- The presence of essential macro (potassium, phosphorus, calcium, sodium and magnesium) and trace minerals (iron, zinc, copper and manganese) have been reported (Arvanitoyannis & Mavromatis, 2009; Sulaiman et al., 2011).
- The presence of phenolic compounds such as flavonoids and its derivatives have also been reported in varying concentration in banana fruit (Bennett et al., 2010; Pereira & Maraschin, 2015).

University of Venda

INTRODUCTION CONTD.

- Phenolic compounds are known for their beneficial health properties in reduction of coronary diseases, inhibition of lipoproteins and antioxidant properties in humans (Vijayakumar et al., 2008; Borges et al., 2014).
- Phenolic compounds reported to be present in banana includes gallic acid, catechins, epicatechins, anthocynanins and other flavonoid derivatives (Bennett et al., 2010; Pereira & Maraschin, 2015).
- According to Freeland-Graves and Trotter (2003), minerals assist in body catalytic, structural and regulatory functions, as electrolytes as well as in cellular and basal metabolism (Freeland-Graves & Trotter, 2003; Sulaiman et al., 2011).
- Physicochemical properties of texture, colour, flavour, pH and nutritive value are properties which the mineral content of a food produce can have a potential effect on (Freeland-Graves & Trotter, 2003).

University of Venda

JUSTIFICATION

- With the beneficial nutrients derived from banana fruit, availability and concentration of these nutrients can be affected by location, climatic factor, agricultural practices, cultural variation and degree of ripeness of the fruit (Del Mar Verde Mendez et al., 2003; Nackz & Shahidi, 2006; Englberger et al., 2010).

- There is however paucity of data on polyphenols and mineral composition of fruits especially bananas cultivated in Limpopo Province of South Africa.

University of Venda

Luvhele

Muomva-red

Mabonde

Williams

OBJECTIVE OF STUDY

- This research therefore seeks to profile the phenolic compounds and mineral content of unripe banana flour (UBF) obtained from commercial and non-commercial banana cultivars.

- Cultivars profiled include one commercial banana cultivar: Williams and three non-commercial banana cultivars: *Luvhele*, *Mabonde* and *Muomva-red* obtained from Limpopo Province of South Africa.

University of Venda

METHODOLOGY


University of Venda

Plant material

- Non-commercial banana cultivars used for this study includes:
  - *Luvhele* (*Musa* ABB),
  - *Mabonde* (*Musa* AAA)
  - *Muomva-red* (*Musa balbisiana*)
  - Williams (commercial banana cultivar)
- obtained at unripe green stage 2 of maturity (Aurore et al., 2009) from household banana farms in Thohoyandou, South Africa.
- Fruit pulp of cultivars was cut to 4 mm size and pretreated with organic acids: ascorbic, citric and lactic acid at concentrations of 10, 15 and 20 g/L for 10 min.
- The pretreated sliced pulp was allowed to drain for 2 min and oven dried in a forced air oven dryer at a temperature of 70°C for 12 h.

University of Venda

Sample preparation

- Oven dried pretreated pulp was used to obtain homogenized banana flour through milling (Retsch ZM 200miller, Haan, Germany) of dried pulp at 16,000 rpm for 30 s.

- UBF obtained from milled unripe banana pulp was then profiled for total polyphenol, antioxidant capacity and individual phenolic compounds.

Mineral analysis

- Analysis for essential and trace elements were measured by a Thermo iCAP 6200 ICP-OES (Thermo Fisher Scientific, Waltham, USA) after calibration and verification with National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) traceable standards.

LC-DAD-ESI-MS analysis of phenolic compounds

- LC–MS analyses of samples was conducted on a Waters Synapt G2 quadrupole time-of-flight mass spectrometer system (Milford, MA, USA). The instrument was connected to a Waters Acquity ultra-performance liquid chromatography (UPLC) and Acquity photo diode array (PDA) detector.

- Gallic acid, catechin, epicatechin and epigallocatechin standards obta from Sigma-Aldrich, South Africa were used for quantification of pher compounds in UBF.

University of Venda

Statistical analysis

- Results of experiment were statistically analyzed with analysis of variance (ANOVA)

- ANOVA was performed using SPSS 22 for windows (SPSS Inc., Chicago, IL).

- Means of obtained results were separated using Duncan Multiple Comparison Test.

University of Venda

RESULT AND DISCUSSION

Mineral profile of UBF

- Organic acid pretreatment showed varying effects with different pretreatment concentrations on all cultivars analysed.
- Ash content of between 1.13 ± 0.05 - 1.33 ± 0.11% had been reported by Anyasi et al. (2015a)
- Zinc had the least occurrence (3.55 – 7.78 mg/kg), while potassium (K) was the most abundant (9117.32 – 14746.73 mg/kg) mineral in UBF of all four banana cultivars.
- Result also showed that essential macro elements had a high level of occurrence in both commercial and non-commercial fruits exar (Table 1).

University of Venda

Table 1. Essential macro mineral profile of UBF obtained from four banana cultivars.

Mineral composition (mg/kg d.w.)	Pretreatment (g/L)								
	Ascorbic			Citric			Lactic		
	10	15	20	10	15	20	10	15	20
Calcium									
Luvhele	208.32 ± 0.51[ef]	221.43 ± 1.03[b]	217.63 ± .0.15[cd]	213.71 ± 1.47[de]	217.35 ± 0.06[cd]	206.47 ± 0.08[f]	214.09 ± 1.04[cde]	220.55 ± 2.28[cd]	245.26 ± 1.00[a]
Mabonde	65.97 ± 1.74[f]	95.59 ± 1.19[d]	114.79 ± 1.09[abc]	80.77 ± 1.02[e]	86.95 ± 1.32[e]	111.12 ± 2.29[bc]	118.84 ± 0.07[a]	108.19 ± 1.31[c]	116.52 ± 0.13[ab]
Muomva-red	237.96 ± 1.28[a]	260.09 ± 2.03[a]	285.08 ± 0.27[a]	220.79 ± 0.33[a]	950.34 ± 0.07[a]	208.12 ± 1.42[a]	242.20 ± 0.21[a]	225.95 ± 0.51[a]	243.64 ± 1.57[a]
Williams	140.15 ± 0.24[c]	103.18 ± 0.58[f]	143.64 ± 1.25[c]	116.17 ± 0.17[e]	124.58 ± 0.55[d]	153.14 ± 1.23[b]	146.28 ± 0.12[bc]	131.48 ± 0.62[d]	162.70 ± 0.17[a]
Magnesium									
Luvhele	897.32 ± 0.22[bc]	913.28 ± 0.04[ab]	899.59 ± 0.18[b]	872.62 ± 0.82[cd]	899.75 ± 0.11[b]	929.29 ± 0.37[a]	893.48 ± 0.65[bc]	867.91 ± 0.61[d]	888.52 ± 0.25[bcd]
Mabonde	876.49 ± 0.29[a]	886.59 ± 0.23[a]	888.08 ± 0.21[a]	843.79 ± 0.50[a]	872.71 ± 0.14[a]	878.69 ± 0.43[a]	879.43 ± 0.44[a]	850.15 ± 0.01[a]	844.61 ± 0.17[a]
Muomva-red	1100.04 ± 0.63[a]	1082.30 ± 0.30[ab]	1102.58 ± 0.53[a]	1048.61 ± 0.10[bc]	1059.60 ± 0.53[abc]	1052.08 ± 0.40[bc]	1072.53 ± 0.51[abc]	1035.48 ± 0.16[c]	1063.57 ± 0.17[abc]
Williams	1149.82 ± 0.16[cd]	1110.07 ± 0.29[ef]	1094.85 ± 0.77[f]	1173.82 ± 0.24[bc]	1098.90 ± 0.25[ef]	1205.53 ± 0.18[ab]	1137.91 ± 0.14[cde]	1127.60 ± 0.40[def]	1229.60 ± 0.77[a]
Phosphorus									
Luvhele	941.67 ± 0.39[ab]	966.89 ± 0.13[a]	909.28 ± 0.20[b]	938.87 ± 0.42[ab]	962.13 ± 0.01[a]	948.17 ± 0.07[ab]	926.02 ± 0.10[ab]	941.93 ± 0.63[ab]	936.63 ± 0.09[ab]
Mabonde	1076.22 ± 0.25[a]	1003.65 ± 0.15[b]	945.27 ± 0.39[c]	996.27 ± 0.93[b]	1017.43 ± 0.06[b]	996.93 ± 0.93[b]	922.50 ± 0.08[cd]	907.79 ± 0.10[cd]	896.93 ± 0.30[d]
Muomva-red	664.10 ± 0.38[a]	680.41 ± 0.18[a]	694.99 ± 0.19[a]	669.53 ± 0.39[a]	667.93 ± 0.03[a]	686.94 ± 0.23[a]	671.87 ± 0.36[a]	661.73 ± 0.00[a]	675.05 ± 0.06[a]
Williams	868.74 ± 0.30[b]	842.89 ± 0.32[b]	954.35 ± 0.58[a]	828.45 ± 0.90[b]	943.30 ± 0.39[a]	931.61 ± 0.13[a]	990.45 ± 0.35[a]	979.58 ± 0.41[a]	946.85 ± 0.33[a]
Potassium									
Luvhele	9229.69 ± 2.62[e]	9700.48 ± 0.49[a]	9490.33 ± 0.60[c]	9227.76 ± 0.33[e]	9596.68 ± 0.33[b]	9675.24 ± 0.32[a]	9117.32 ± 0.61[f]	9050.05 ± 0.11[g]	9426.69 ± 0.60[d]
Mabonde	14746.73 ± 1.69[a]	13984.33 ± 1.82[b]	13213.48 ± 0.21[e]	13523.72 ± 0.62[d]	13850.63 ± 2.65[c]	13557.67 ± 0.82[d]	12371.30 ± 0.93[f]	13112.69 ± 1.50[e]	12181.91 ± 2.26[g]
Muomva-red	12317.01 ± 2.15[b]	12464.86 ± 0.34[a]	12292.32 ± 0.64[b]	11742.70 ± 1.79[e]	12422.66 ± 1.71[ab]	12484.17 ± 0.19[a]	11915.20 ± 1.47[d]	12054.34 ± 1.05[c]	11397.51 ± 0.29[f]
Williams	11375.42 ± 0.53[b]	12030.64 ± 0.20[a]	10404.01 ± 1.32[e]	10817.57 ± 0.14[c]	10855.88 ± 0.82[c]	10499.60 ± 0.83[d]	10825.51 ± 0.43[c]	10583.44 ± 1.35[d]	10377.49 ± 1.30[e]
Sulphur									
Luvhele	333.41 ± 0.15[a]	337.13 ± 0.16[a]	328.17 ± 0.17[a]	333.65 ± 0.65[a]	342.76 ± 0.27[a]	334.07 ± 0.46[a]	333.59 ± 0.00[a]	333.33 ± 0.28[a]	347.36 ± 0.13[a]
Mabonde	361.25 ± 0.43[a]	361.21 ± 0.19[a]	335.21 ± 0.22[a]	348.19 ± 0.48[a]	354.04 ± 0.62[a]	343.92 ± 0.15[a]	338.15 ± 0.06[a]	341.64 ± 0.19[a]	331.62 ± 0.02[a]
Muomva-red	369.51 ± 0.80[a]	348.38 ± 0.19[a]	354.12 ± 0.15[a]	343.37 ± 0.07[a]	349.57 ± 0.32[a]	345.21 ± 0.02[a]	366.25 ± 0.75[a]	350.64 ± 0.05[a]	359.13 ± 0.05[a]
Williams	338.90 ± 0.02[ab]	335.31 ± 0.37[abc]	323.69 ± 0.24[bc]	318.34 ± 1.11[c]	335.83 ± 0.25[abc]	339.11 ± 0.70[a]	339.72 ± 0.57[ab]	339.64 ± 0.09[ab]	349.67 ± 0.05[a]

Means with different letters across rows are significantly different ($p < 0.05$) using Duncan multiple comparison test. Values are means ± SE of triplicate measurements.

- Occurrence of essential trace elements was generally low when compared to the results obtained for essential macro elements.
- Results showed that iron (Fe) recorded a higher concentration of 8.15 - 33.72 mg/kg than zinc (Zn) with a concentration of between 3.55 - 7.78 mg/kg in all cultivars examined and across pretreatment concentration (Table 2).

Table 2. Essential trace mineral profile of UBF obtained from four banana cultivars.

Mineral composition (mg/kg d.w.)	Pretreatment (g/L)								
	Ascorbic			Citric			Lactic		
	10	15	20	10	15	20	10	15	20
Iron									
Luvhele	32.35 ± 0.52[b]	11.01 ± 0.36[e]	11.28 ± 0.78[e]	13.76 ± 1.18[de]	13.36 ± 0.43[de]	20.05 ± 0.84[c]	39.72 ± 0.68[a]	15.92 ± 0.76[d]	11.99 ± 2.85[de]
Mabonde	25.62 ± 0.93[a]	16.27 ± 1.72[b]	16.41 ± 2.49[b]	9.55 ± 0.85[c]	10.99 ± 2.27[c]	9.98 ± 3.14[c]	8.85 ± 1.72[c]	11.26 ± 0.59[c]	9.62 ± 0.16[c]
Muomva-red	15.54 ± 0.32[c]	32.49 ± 0.34[a]	33.06 ± 1.07[a]	15.16 ± 0.05[c]	21.19 ± 0.77[b]	33.72 ± 1.06[a]	13.98 ± 2.31[c]	18.43 ± 0.32[bc]	14.03 ± 1.60[c]
Williams	8.79 ± 3.31[b]	9.65 ± 0.58[ab]	8.44 ± 1.58[b]	8.92 ± 1.56[b]	8.15 ± 0.81[b]	9.96 ± 2.24[ab]	13.70 ± 0.19[a]	9.29 ± 3.71[b]	8.69 ± 0.89[b]
Zinc									
Luvhele	7.78 ± 2.90[a]	6.89 ± 1.50[b]	6.34 ± 0.80[cd]	6.86 ± 4.40[b]	6.57 ± 0.60[bc]	6.55 ± 2.50[bc]	6.86 ± 2.10[b]	5.97 ± 2.70[e]	6.10 ± 1.10[de]
Mabonde	3.96 ± 3.40[de]	4.15 ± 0.70[cd]	4.29 ± 0.80[c]	4.75 ± 1.80[b]	5.08 ± 3.00[a]	4.41 ± 0.40[c]	4.73 ± 1.90[b]	3.91 ± 4.40[de]	3.73 ± 2.60[e]
Muomva-red	4.70 ± 0.60[a]	4.39 ± 3.80[b]	4.31 ± 5.00[b]	3.97 ± 0.60[c]	3.97 ± 3.40[c]	3.97 ± 3.80[c]	3.88 ± 3.80[c]	3.99 ± 0.70[c]	3.87 ± 6.00[c]
Williams	3.72 ± 2.40[cd]	3.69 ± 4.70[cd]	3.93 ± 1.30[c]	3.68 ± 7.30[cd]	3.55 ± 2.00[d]	4.45 ± 4.50[b]	3.96 ± 5.20[c]	4.82 ± 3.10[a]	4.60 ± 5.30[ab]

Means with different letters across rows are significantly different ($p < 0.05$) using Duncan multiple comparison test. Values are means ± SE of triplicate measurements.

Profile of phenolic compounds present in UBF

- Total phenolic content of UBF from non-commercial banana cultivars had earlier been reported to be in the range of 841.59 ± 38.39 - 1130.39 ± 27.26 mg GAE/100 g d.w. (Anyasi et al. 2015b).
- Gallic acid, catechin, epicatechin, epigallocatechin and myricetin-O-rhamnoside were phenolic compounds that were analysed in UBF.
- Of the phenolic compounds examined, catechin, gallic acid and epigallocatechin were not detected in both commercial and non-commercial UBF.
- Although several authors have reported the presence of gallic acid and catechin in pulp and peel of both ripe and unripe banana cultivars obtained from other regions of the world (Bennett et al., 2010; Borges et al., 2014).
- However, epicatechin (Fig. 1) and myricetin-O-rhamnoside (Fig. 2) were detected in varying concentrations in UBF of banana samples examined.

University of Venda

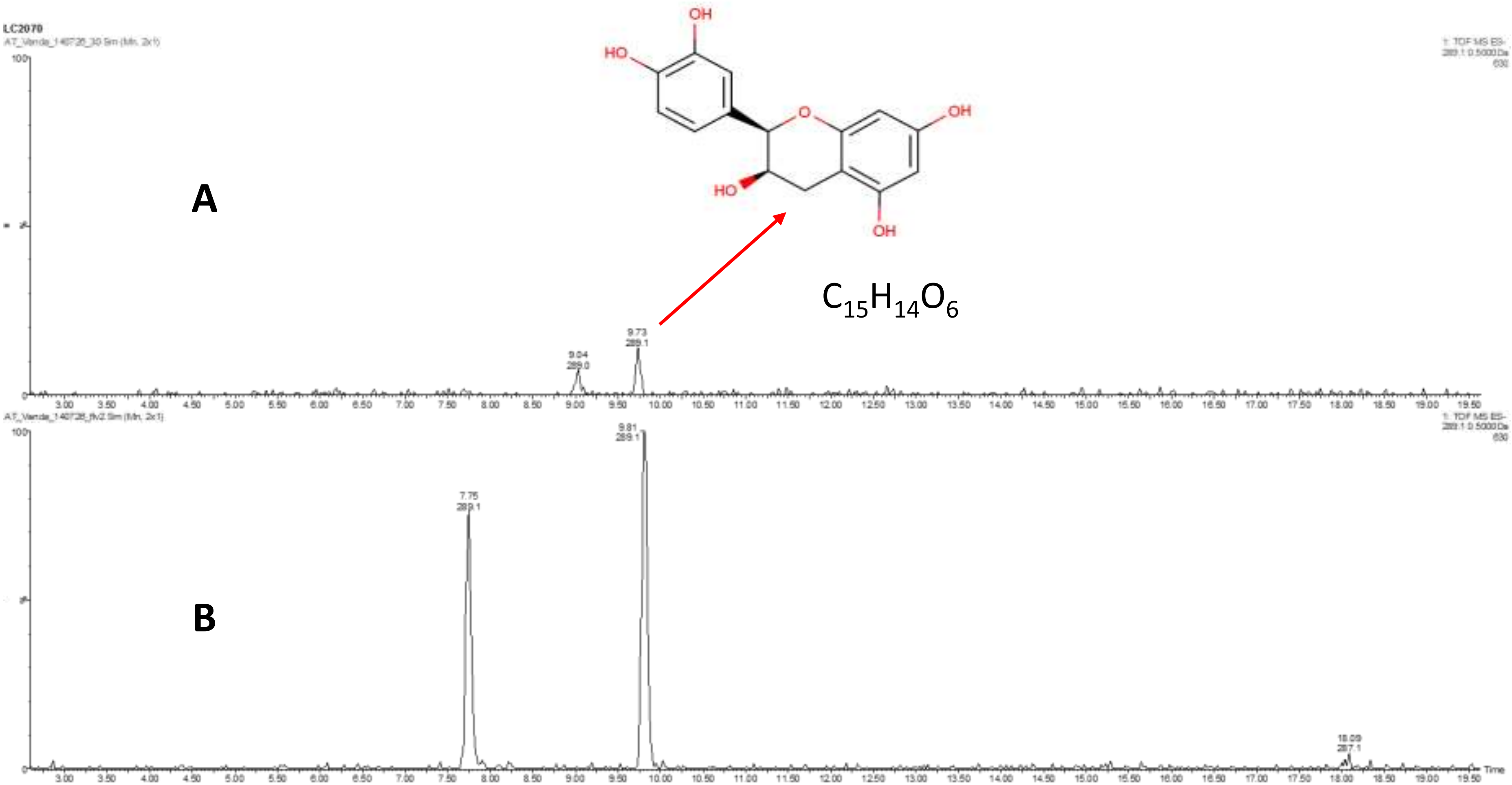

Figure 1. Extracted ion chromatograms (m/z 289; [M-H]- ion of epicatechin), A, citric acid pretreated (20 g/L) UBF; B, 1.2 ppm epicatechin standard.

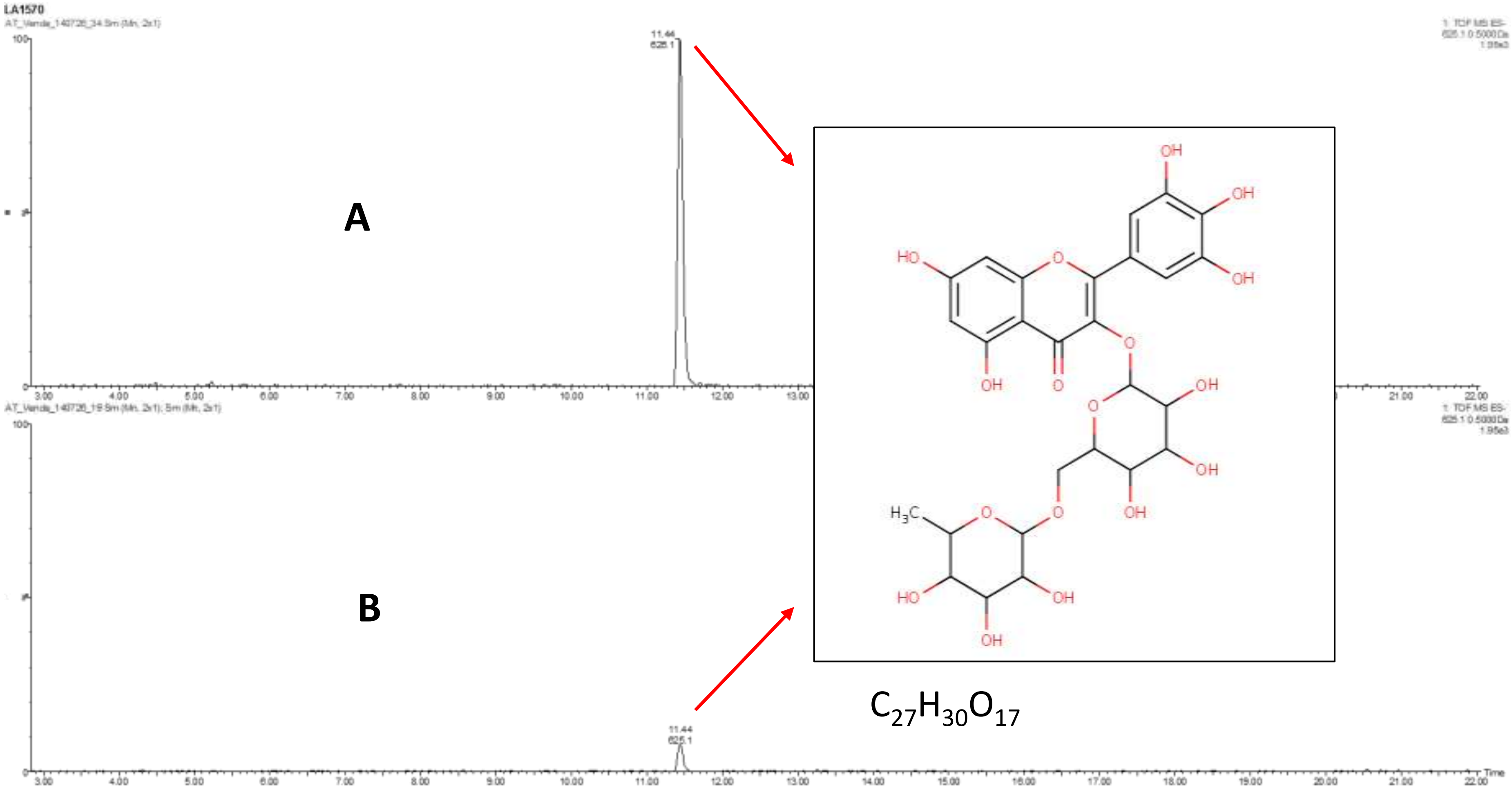

Figure 2. Extracted ion chromatograms of myricetin-O-rhamnoside. **A:** ascorbic acid pretreated (15 g/L) UBF; **B:** lactic acid pretreated *Muomva-red* (15 g/L) UBF.

Table 3. Polyphenol concentration of organic acid pretreated UBF.

Phenolic compound (µg/g d.w.)	Pretreatment (g/L)								
	Ascorbic			Citric			Lactic		
	10	15	20	10	15	20	10	15	20
Epicatechin									
Luvhele	2.73 ± 0.50^{a}	3.56 ± 0.69^{a}	3.49 ± 0.67^{a}	4.13 ± 0.83^{a}	2.80 ± 0.48^{a}	4.05 ± 0.76^{a}	2.27 ± 0.41^{a}	2.47 ± 0.40^{a}	4.24 ± 0.84^{a}
Mabonde	nd	nd	1.35 ± 0.18^{a}	nd	nd	nd	nd	nd	nd
Muomva-red	1.36 ± 0.14^{ab}	1.66 ± 0.24^{a}	1.42 ± 0.19^{ab}	1.11 ± 0.10^{b}	1.73 ± 0.23^{a}	1.06 ± 0.10^{b}	nd	1.01 ± 0.08^{b}	nd
Williams	1.62 ± 0.22^{b}	1.67 ± 0.22^{b}	3.24 ± 0.58^{a}	1.40 ± 0.19^{b}	nd	1.47 ± 0.19^{b}	1.30 ± 0.15^{b}	1.27 ± 0.15^{b}	nd
Myricetin-O-rhamnoside									
Luvhele	17.33 ± 2.31^{a}	18.01 ± 3.23^{a}	17.08 ± 1.97^{a}	16.02 ± 1.21^{ab}	18.04 ± 1.74^{a}	13.91 ± 1.61^{bc}	12.52 ± 2.24^{ab}	13.19 ± 1.50^{ab}	9.91 ± 1.58^{b}
Mabonde	6.37 ± 1.14^{bc}	8.33 ± 1.33^{b}	13.69 ± 1.61^{a}	4.60 ± 0.77^{cd}	2.60 ± 0.27^{d}	5.68 ± 1.04^{bc}	7.20 ± 0.58^{bc}	1.93 ± 0.10^{d}	8.20 ± 0.96^{b}
Muomva-red	10.81 ± 0.81^{ab}	11.59 ± 0.64^{ab}	11.21 ± 0.84^{ab}	12.26 ± 0.53^{a}	11.27 ± 1.52^{ab}	11.01 ± 0.19^{ab}	12.19 ± 0.40^{a}	11.97 ± 0.10^{ab}	9.51 ± 0.98^{b}
Williams	3.76 ± 0.59^{c}	5.74 ± 0.34^{b}	7.61 ± 0.82^{a}	1.92 ± 0.19^{d}	2.00 ± 0.23^{d}	3.64 ± 0.36^{c}	2.88 ± 0.19^{cd}	4.20 ± 0.15^{c}	3.39 ± 0.40^{c}

Means with different letters across rows are significantly different ($p < 0.05$) using Duncan multiple comparison test. Values are means ± SE of triplicate measurement.
nd not detected

- Occurrence of myricetin-O-rhamnoside was significantly different ($p < 0.05$) in all UBF of both commercial and non-commercial varieties.
- It was observed that the non-commercial UBF had higher concentration of myricetin-O-rhamnoside when compared to the commercial UBF.

- *Luvhele* UBF recorded the highest concentration (17.33 ± 2.31 µg/g) of myricetin-O-rhamnoside, at ascorbic acid pretreatment concentration of 10 g/L while Williams UBF recorded the least concentration (1.92 ± 0.19 µg/g) at citric acid pretreatment of 10 g/L.
- Myricetin-O-rhamnoside at m/z 625.1 is a flavonoid derivative which has not been reported in literature to be present in banana pulp.
- According to Regazzoni et al. (2013), the compound was only reported to be present in aqueous extract of sumac leaves (*Rhus coriaria* L.) when determined by flow injection analysis with high-resolution mass spectrometry.
- Abu-Reidah et al. (2015) also reported the presence of myricetin-O-rhamnoside in epicarp of sumac fruit when the compound was analysed using HPLC-DAD-ESI-MS/MS.

University of Venda

CONCLUSION AND RECOMMENDATION

- Non-commercial banana cultivars obtained from Limpopo Province of South Africa are a good source of essential macro and trace elements.
- Conversely, there was generally low concentration of polyphenols in UBF when compared to cultivars obtained from other parts of the world.
- Phenolic compounds such as gallic acid and catechin reported to be present in cultivars obtained in different parts of the world were not detected in UBF used for this analysis.
- According to Lazarte et al. (2015), bioavailability of mineral nutrients can be affected by the presence of phytochemicals in fruits hence reducing absorption and body utilization.
- Phytate levels of food produce can therefore be investigated in order to determine their effect in terms of bioavailability of these mineral nutrients in the body.

University of Venda

ACKNOWLEDGEMENT

The authors wish to acknowledge the financial contributions and support to Tonna Anyasi from the following

- Prof A.I.O. Jideani
- DST-NRF Centre of Excellence in Food Security
- University of Venda Research and Publication Committee Research Fund SARDF/13/FST/01
- Work Study Program of the University of Venda, South Africa.

University of Venda

REFERENCES

- Abu-Reidah, I. M., Ali-Shtayeh, M. S., Jamous, R. M., Arráez-Román, D., & Segura-Carretero, A. (2015). HPLC–DAD–ESI-MS/MS screening of bioactive components from *Rhus coriaria* L. (Sumac) fruits. *Food Chemistry, 166,* 179–191.

- Arvanitoyannis, I. S., & Mavromatis, A. (2009). Banana cultivars, cultivation practices, and physicochemical properties. *Critical Reviews in Food Science and Nutrition, 49,* 113-135.

- Bennett, R. N., Shiga, T. M., Hassimotto, N. M. A., Rosa, E. A. S., Lajolo, F. M. & Cordenunsi, B. R. (2010). Phenolics and antioxidant properties of fruit pulp and cell wall fractions of postharvest banana (*Musa acuminata* Juss.) cultivars. *Journal of Agricultural and Food Chemistry, 58,* 7991-8003.

- Borges, C. V., Amorim, V. B. O., Ramlov, F., Ledo, C. A. S., Donato, M., Maraschin, M., & Amorim, E. P., (2014). Characterisation of metabolic profile of banana genotypes, aiming at biofortified *Musa* spp.cultivars. *Food Chemistry, 145,* 496–504.

- Facundo, H. V. D. V., Gurak, P. D., Mercadante, A. Z., Lajolo, F. M., & Cordenunsi, B. R. (2015). Storage at low temperature differentially affects the colour and carotenoid composition of two cultivars of banana. *Food Chemistry,* 170, 102–109.

- Lazarte, C. E., Carlsson, N-G., Almgren, A., Sandberg, A-S., & Granfeldt, Y. (2015). Phytate, zinc, iron and calcium content of common Bolivian food, and implications for mineral bioavailability. *Journal of Food Composition and Analysis, 39,* 111–119.

University of Venda

THANK YOU!