

Preliminary Assessment of the Antihypertensive and Antioxidative Activities of the Peptides from “Saba” Banana (*Musa balbisiana Colla*) Flesh

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ABSTRACT

The crude proteins were isolated using 0.125 M Tris-HCl with 50 mM NaCl at pH 7.4. The protein content of the crude extract was determined using the Lowry assay and was found to be 4.28 mg/mL. The major band which corresponds to the major protein has an approximate molecular weight of 20 KDa. The isolated crude proteins were subjected to enzymatic digestion using pepsin, trypsin, chymotrypsin, and thermolysin for 3, 4, 12, and 24 hours. The 24-hour digest was found to have the highest percent anti-Angiotensin Converting Enzyme (ACE) activity (36.02%) while the 12-hour digest was found to have the highest anti-oxidative activity (33.14%) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The 24-hour digest was subjected to Ultra-Performance Liquid Chromatography (UPLC) to determine the peptide fraction responsible for the ACE inhibitory activity. Three peptide fractions (PF1, PF2 and PF3) were chosen and PF2 exhibited the highest percent inhibitory activity (32.21) against ACE. PF2 was subjected to thin layer chromatography (TLC) and the possible identity is EK based on the R_f value traveled by glutamic acid (E) (0.43) and Lysine (K) (0.13). *In silico* analysis was done to correlate the results with the presence of putative peptides with antioxidative and antihypertensive activities. Results showed that antihypertensive peptides EK, GS, TY, FNE, FP, LKA, PT, PP, FAL and antioxidative peptides IR and VPW were found based on the sequence of the protein in “Saba” banana. The presence of the antihypertensive peptide EK was verified using thin layer chromatography (TLC).

Keywords: *Saba banana; Crude Proteins; peptides; ACE*

INTRODUCTION

There have been numerous studies on alternative ways on how to treat some diseases in a natural, cheap and safe way. Of these diseases, hypertension and the diseases caused by oxidative stress are the two being dealt with. Hypertension is usually defined by the presence of a chronic elevation of systemic arterial

pressure above a certain threshold value (Giles *et al.*, 2009). According to Chopra and Ram (2019), the latest European guidelines regarding hypertension sets the threshold value of blood pressure to be 140/90 mm Hg while the American guidelines lowered the threshold to 130/80 mm Hg. According to the World Health Organization (WHO) data published in 2017, hypertension death rate in the

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Philippines reached 23.56 per 100,000 population, and is ranked number 44 all over the world. In 2019, WHO indicated that hypertension is estimated to cause 7.5 million deaths worldwide.

Oxidative stress is essentially an imbalance in the production of reactive oxygen species (ROS) and antioxidants in the body. Studies show that the production of ROS may affect hypertension and mechanisms involved are not yet well understood. Loperena and Harrison in 2017 described three probable mechanisms on how ROS is linked to hypertension. Since ROS can be produced in the kidneys, vasculature and the central nervous system (CNS), these three sites come with different mechanisms. For renal oxidative stress, increase in the ROS leads to a chain of reactions that promote vasoconstriction and sodium (Na) retention, both of which leads to hypertension. On the other hand, increase of ROS in blood levels may provide sets of signaling phenomena and reactions that may lead to blood vessel narrowing via vasoconstriction, vascular smooth muscle hypertrophy (luminal narrowing) or vascular fibrosis (collagen deposition). In the case of oxidative stress in CNS, this will lead to stimuli production that affects the kidney and also the blood vessels.

Bioactive peptides are defined as food-derived components which may be genuine or synthetically generated that exert a physiological effect in the body like promoting human health by reducing the risk of chronic diseases and boosting natural immune protection in addition to their nutritional value (Korhonen and Pihlanto, 2006). Bioactive peptides have already been observed from different crops including the legumes such as soybean and mungbean (Cabanos *et al.*, 2012), and studying other crops may add to the growing spectrum of alternative ways of health improvement.

Bananas are common and can provide a lot of health benefits. Bananas are known to be rich on carbohydrates and dietary fibers (Sidhu & Zafar, 2018), vitamins (A, B6, C, D) and minerals (Kumar *et al.*, 2012). It is a good source of potassium which may reduce the risk of hypertension and stroke as shown in the cross-sectional study of Dayanand and colleagues in 2015 showing a significant decrease in the blood pressure levels of human subjects. Pereira and Maraschin (2014) and Singh *et al.* (2016) reported that bananas are rich in many bioactive compounds such as carotenoids, flavonoids, phenolics, and amines which have antioxidant activities.

“Saba” banana is one of the major banana cultivars in the Philippines in addition to “Lakatan” and

“Latundan”. Many of the studies in “Saba” banana focuses on its yield and disease resistant. There has been no published reports yet for its proteins and peptides and their bioactivities. This research is the first attempt to report on the proteins and peptides with antihypertensive and antioxidative activities in “Saba” banana.

The general objective of this study is to assess the peptides found in “Saba” banana (*Musa balbisiana* Colla) flesh for the presence of bioactive peptides with antioxidative and antihypertensive activities.

MATERIALS AND METHODS

Summary of Materials. “Saba” banana fruits were acquired from a local store in Brgy. Tuntungin-Putho, Los Baños, Laguna, Philippines. Fresh pig lungs were obtained from the Los Baños Community Market, Los Baños, Laguna, Philippines. Captopril (Capomed™) tablets (25 mg/tablet) were bought from a local drugstore. All other reagents used in this experiment were analytical grade.

Isolation of “Saba” Banana Crude Protein Extract. The method of Esteve *et al.* (2012) with modifications was used for the crude extract isolation. Three fingers of “Saba” banana flesh were ground using mortar and pestle. Liquid nitrogen was added to further pulverize the ground flesh sample. About 180 mL of non-denaturing extraction buffer containing 0.125 M Tris-HCl (pH 7.4) and 50 mM NaCl was added to the pulverized sample. The resulting mixture was placed in an ice bath, and then stirred 12 h using a magnetic stirrer. Then, the resulting mixture was centrifuged using an Allegra X-30R Refrigerated Centrifuge (Beckman Coulter, USA) at 4°C, 12,000rpm for 30 min. The clarified supernatant was considered the crude protein extract from “Saba” banana flesh, which was kept in cold storage (-20°C) until use.

Protein Content Determination by Lowry Method. Protein concentration was determined using a modified method described by Lowry *et al.* (1951), with bovine serum albumin (BSA) as protein standard.

In vitro Enzymatic Digestion of the “Saba” Banana Crude Protein Extract. In vitro enzymatic digestion of the crude protein extract was done separately using pepsin, trypsin, α -chymotrypsin and thermolysin.

Hydrolysis was done at different time intervals (0 h, 4h, 12 h and 24 h) according to specific conditions that optimize the activity of each enzyme as described by Contreras *et al.* (2009) and Marczak *et al.* (2003),

with some modifications. The progress of enzymatic digestion was monitored through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

For pepsin, 200 μ L aliquots of 1.0 mg/mL crude protein, suspended in 35 mM phosphoric acid-potassium dihydrogen phosphate buffer [(pH 2.0) with 0.4 M NaCl and 0.02% NaN₃], were made for each time interval. Then, 25 μ L of 1.0 mg/mL of pepsin was added onto each aliquot. Digestion was stopped by bringing the digests to neutralization using 1.0 M NaOH and heating the digests in a boiling water bath for 5 min. The digests were immediately stored in ice.

For trypsin, α -chymotrypsin and thermolysin, 200 μ L aliquots of 1.0 mg/mL crude protein were obtained to which 25 μ L of 1.0 mg/mL of the enzyme was added for each time interval. Digestion was stopped by heating the hydrolysates in a boiling water bath for 5 min. The digests were immediately stored in ice.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE was performed as described by the method of Laemmli (1970). Electrophoresis was accomplished on 15% discontinuous denaturing gels using a Mini-PROTEAN® Tetra Cell electrophoresis apparatus (Bio-Rad, USA). The electrophoresis set-up was run at 110 V for 1.5 h. After the run, gels were stained with an aqueous solution containing 0.05% (w/v) Coomassie Brilliant Blue R250, 50% (v/v) methanol and 10% (v/v) acetic acid. Then, the gels were destained sequentially with an aqueous solution of 50% (v/v) methanol and 10% (v/v) acetic acid, and an aqueous solution of 5% (v/v) methanol and 7% (v/v) acetic acid. Benchmark Protein™ Unstained Standards (Bio-Rad, USA) for SDS-PAGE was used as molecular weight markers to determine the size of the protein.

Densitometric Analysis. Analysis of the percent composition of the different proteins was done on the scanned images of the electrophoretograms using the TotalLab® high-quality image analysis software.

Extraction of ACE from Pig Lungs. The method described by Folk *et al.* (1960) was used to extract ACE from pig lungs, with slight modifications. One hundred (100) grams of fresh pig lungs were obtained, cut into pieces, and then minced. The minced tissue was defatted and then homogenized sequentially with acetone (1:2 w/v) and diethyl ether (1:2 w/v). The defatted tissue was air dried, then ground into powder. The tissue powder was suspended in 100 mM sodium borate buffer (pH 8.3)

at 1:5 w/v, then mixed for 3 h at 4°C. The mixture was filtered through four layers of cheesecloth. The resulting supernatant was centrifuged at 10,000 rpm for 30 min, stored and labeled as the ACE extract.

ACE Inhibition Assay. The method for assessing the antihypertensive property of the protein hydrolysates was adapted from Cushman *et al.* (1977), as described by Dumandan *et al.* (2014), with slight modifications.

Assay mixtures (triplicates per digestion time 2h, 4h, 12h, and 24h) containing 25 μ L of 1.0 mg/mL protein digest, 25 μ L 100 mM phosphate buffer, 25 μ L 300 mM NaCl, and 50 μ L of 5 mM N-hippuryl-L-histidyl-L-leucine (HHL) (Sigma-Aldrich, USA), were placed in clean test tubes. A negative control was prepared by adding 125 μ L 1.0 M HCl to an assay mixture. A blank was also prepared by adding 25 μ L of the ACE extract to an assay mixture. Samples were prepared by adding 25 μ L of the protein hydrolysate (from each time interval for each enzyme) to an assay mixture, and then 25 μ L of the ACE extract were subsequently added. A positive control was also prepared using 1.0 mg/mL captopril. The addition of the ACE extract to the blank, samples, and control initiated the enzyme-catalyzed reaction.

The mixtures were then incubated at 37°C for 30 min. Except for the negative control, 125 μ L of 1.0 M HCl was added into the mixtures to terminate the enzyme reaction. The hippuric acid (HA) formed was extracted by adding 750 μ L of ethyl acetate. From each tube, a 500 μ L aliquot of the ethyl acetate layer was collected and evaporated to dryness. Hippuric acid was dissolved in 500 μ L distilled water and its absorbance was read at 228 nm.

The percent inhibition of the protein digest from ACE was calculated according to the equation described by Cushman *et al.* (1977).

$$\% \text{ Inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}} - A_{\text{blank}}} \right) \times 100$$

where A_{blank} is the absorbance of blank at 228 nm, A_{sample} is the absorbance of the sample at 228 nm, and A_{control} is the absorbance of the negative control at 228 nm.

Determination of the Radical Scavenging Activity by 2,2-diphenylpicrylhydrazyl (DPPH) Assay. The method of Saha *et al.* (2008) for the DPPH assay using ascorbic acid as standard was used for all crude proteins and protein hydrolysates samples.

Fractionation of Protein Digests Using Ultra-Performance Liquid Chromatography (UPLC).

The method of Silva *et al.* (2006) with some modifications for all protein digests was used. The protein digests with the highest activity for ACE inhibition was used for the analysis. UPLC was performed using the Acquity UPLC RP-C18 column (2.1 x 50 mm, 1.7 μ m bead) eluted with gradient 1 at flow rate of 0.6 mL/min and absorbance monitored at 280 nm. An aqueous solution (80%) acetonitrile was used as the solvent system in the analysis. About 10 μ L of the 1.0 mg/ml digested sample was injected into the reversed-phase column with a guard column and disposable cartridge (10mm, 12 μ m). The elution profile was as follows: temperature at 40 °C via a mobile phase of two solvents—solvent A: 0.1% (vol/vol) TFA in water, and solvent B: 0.1% (vol/vol) TFA in 80% acetonitrile:water (9:1)— at a flow rate of 1.0 mL/min, starting with 98% (vol/vol) solvent A, passing through a linear gradient from 2 to 60% (vol/vol) solvent B over 45 minutes, another linear gradient from 60% to 100% (vol/vol) solvent B for 2 minutes, 100% (vol/vol) solvent B for 5 minutes, and a linear gradient from 98 to 2% (vol/vol) solvent B for 2 minutes, and ending with 2% (vol/vol) solvent B for 2 minutes.

The detection was read at 215 nm using a spectrophotometer. The fractions that were collected were based upon their peak assignments. Collected fractions was subjected to spectrophotometric assay of ACE activity described earlier.

Characterization of the Peptide Amino Acid Composition Obtained from Ultra-Performance Liquid Chromatography Using Thin Layer Chromatography (TLC).

The standard amino acids were dissolved in 0.01 M sodium hydroxide solution to a final concentration of 0.01 M. The mixture of butanol, acetic acid, and water in a 5:3:2 ratio with 0.4 % w/v ninhydrin was prepared as the developing solvent. The TLC plates were prepared using silica as the stationary phase. Using a 10- μ L pipettor, the standard amino acids and the base-hydrolyzed samples were carefully spotted to the prepared TLC plates. After spotting, the plates were placed in the developing chamber containing the developing solvent previously prepared. After the solvent front has reached 3-mm mark before the end of the stationary phase, the plates were then dried for color yield by placing them in an oven at 90 °C for about five minutes. Retention factors (R_f) of the standard amino acids and the samples were then measured.

In Silico Analysis of the “Saba” Banana Major Protein. The amino acid sequence of the “Saba” banana major protein was obtained from <http://www.uniprot.org/> and the bioactive peptides exhibiting

anti-oxidative and anti-hypertensive activities were determined using the protein sequence analyzer in <http://www.uwm.edu.pl/biochemia/index.php/en/biopep>.

Statistical Analysis. Assays were done in duplicates, and subjected to ordinary one-way ANOVA followed by Tukey’s test at 95% confidence interval using GraphPad Prism®. Assay results were expressed as mean activity \pm standard deviation.

RESULTS AND DISCUSSION

“Saba” Banana Protein Isolation. Flesh from three ripe “Saba” bananas was used for the isolation of the crude proteins because according to Mohapatra *et al.* (2010) the protein content increases as the fruit matures.

Results showed that the crude protein is in the soluble fraction (Figure 1).

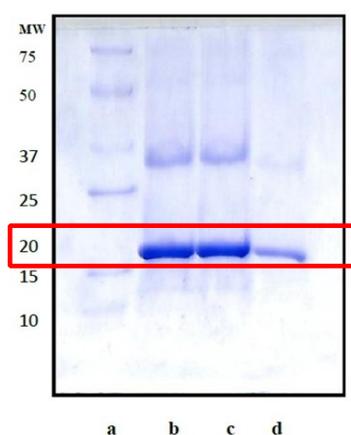


Figure 1. SDS-PAGE profiles for the isolated crude protein from “Saba” banana flesh. Legend: a) protein marker; b) crude protein extract; c) soluble crude protein component; and d) insoluble crude protein component. The electrophoresis was done in a 15% and run at 110 V for 100 minutes.

Solubility of the crude protein in the buffer used is crucial because further analysis of the proteins and peptides require using a solution instead of precipitate or colloidal mixture. Undissolved particles may hinder or interrupt the analysis of proteins and peptides. Two major bands appeared in the electropherogram gel corresponding to approximate molecular weights of 35 KDa and 20 KDa. Between the two major bands, the 20 KDa proteins is more prominent as indicated in the densitometric analysis (Figure 2).

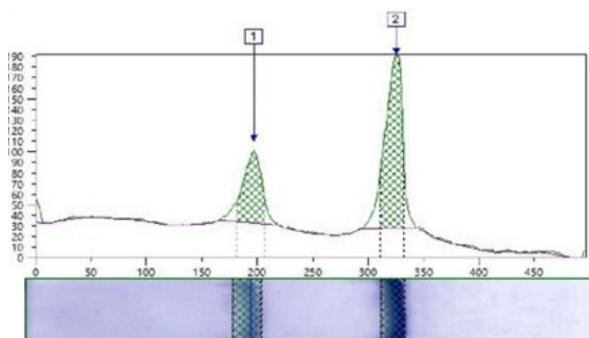


Figure 2. Densitometric Analysis of the SDS-PAGE Profile of the Crude Protein from "Saba" Banana Flesh.

The concentration of the crude protein was also determined using the Lowry method and found to be 4.28 mg/mL. This assay is specific for the amount of protein only and not of the peptide. The protein concentration is important to protein digestion and other characterization assays because most of the methods employed in this study requires a protein concentration not lower than 1.0 mg/mL

Protein Digestion. Pepsin, trypsin, chymotrypsin and thermolysin were used for in vitro simulation of the crude protein present in the stomach and small intestine. Pepsin hydrolyzes proteins at peptide bonds on the amino-terminal side of the amino acid residues Phenylalanine, Tryptophan, and Tyrosine cleaving long peptide chains into a mixture of smaller peptides. Trypsin cleaves peptide bond involving the carboxyl side of the basic amino acids Arginine and Lysine (Stryer, *et al.*, 2002). On the other hand, chymotrypsin cleaves a peptide bond involving the carboxyl side of aromatic amino acids Phenylalanine,

Tryptophan, and Tyrosine and Leucine (Stryer, *et al.*, 2002). Lastly, Thermolysin cleaves a peptide bond involving the amino side of aromatic amino acids Phenylalanine, Tryptophan, and Tyrosine and amino acids with bulky non-polar side chains Leucine, Isoleucine and Valine.

The protein is digested first using pepsin then followed by the three other enzymes to mimic digestion in the stomach and later to the small intestine. Digestion using pepsin was done for two hours at acidic pH (pH 2.0) to simulate digestion in the stomach. After two hours of pepsin digestion, the peptic digest is subjected to enzymatic digestion by trypsin, chymotrypsin and thermolysin. The pH adjustment is necessary to achieve at least the average optimum pH of the three digestive enzymes. Trypsin is formed and takes action in the small intestine when the inactive form trypsinogen produced by the pancreas is activated. Chymotrypsin also takes action in the intestine as the inactive form chymotrypsinogen

produced by the pancreas is activated (Lehninger & Nelson, 2008). Thermolysin on the other hand is a metalloendopeptidase belonging to class metalloproteases which contain a metal ion at the active site which acts as a catalyst in the hydrolysis of the peptide bonds.

Antihypertensive Activity. The antihypertensive activity was determined using Angiotensin Converting Enzyme (ACE) inhibition assay. Results showed that all digests at different digestion times yielded ACE inhibition activities. It was observed that the percent inhibition increases as digestion proceeds (Figure 4).

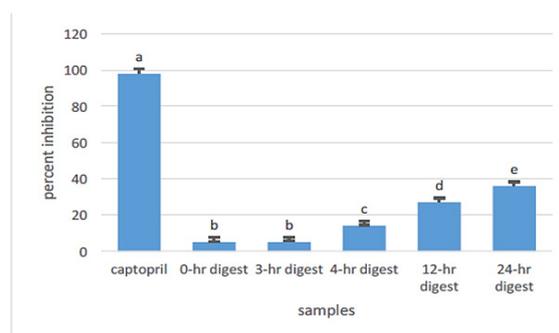


Figure 3. ACE-inhibitory Activities of Captopril and the "Saba" Crude Protein Digests at Different Digestion Time.

Possible explanation for this is that as the digestion progresses, more peptides with antihypertensive activity are released. Among the different digested samples, the 24-hour digest showed the highest percent ACE inhibition (36.02). According to the study conducted by Hernandez-Ledesma *et al.* (2011), the majority of the ACE inhibitory peptides have relatively short sequences, ranging from 2 to 12 amino acids, because the inhibition site for ACE is relatively small thus cannot accommodate larger peptide molecules. In addition, Hyun and Shin (2000) had observed that ACE inhibitory peptides are effectively released after 24 h of digestion or when digestion of the protein is almost complete. The research group has also tried to use crude protein from the boiled sample but no percent inhibition was observed (data not shown). This shows that boiling affects the structure as well as the digestion of the protein. It implies that boiling aids in the complete digestion of the protein not as peptides but into individual amino acid units. Thus, no antihypertensive activity was observed.

Antioxidative Activity. The antioxidative activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) which determines the radical scavenging activity of the peptides against DPPH.

Results showed that all of the digests exhibited free radical scavenging activity with the 12-hour digests giving the highest free radical scavenging activity (33.14%) (Figure 5).

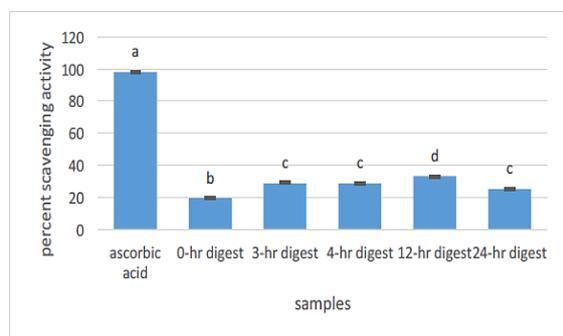


Figure 4. Radical Scavenging Activities of Ascorbic acid and the “Saba” Crude Protein Digests at Different Digestion Time Determined through DPPH Assay.

This suggests that peptides with potential antioxidative activity are released after 12 hours of digestion. However, ascorbic acid which is the standard used for this assay and a known natural antioxidant, had higher antioxidant activity compared to all digests. In the study conducted by Abu-Salem *et al.* (2013), they reported that low molecular weight peptides generally exhibit high antioxidant activities. Digestion of proteins favored the generation and release of potent antioxidant peptides (Bamdad *et al.*, 2011; Chanput *et al.*, 2009). The result obtained in this study proved that aside from tannins and other phenolic compounds such as anthocyanin, delphinidin and cyanidin present in banana (Fatemeh *et al.*, 2012), the peptides may also add to the antioxidative properties of the said fruit.

Fractionation of Protein Digest using Ultra-Performance Liquid Chromatography (UPLC).

The protein digests with the highest percent (%) ACE inhibition was subjected to Ultra-Performance Liquid Chromatography (UPLC) for further analysis of the peptides that may exhibit antihypertensive property. Three peptide fractions corresponding to three highest peaks (Figure 5) were considered for further analysis using ACE assay.

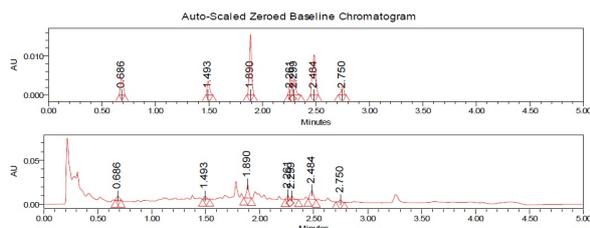


Figure 5. Normal and Auto-scaled Zeroed Baseline Chromatogram of the 24-hour Purified Unboiled “Saba” Banana Flesh Protein (Pectinesterase) upon Fractionation in Ultra-Performance Liquid Chromatography (UPLC).

The three peptide fractions were those eluted at 1.493 minutes (Peptide Fraction 1, PF1), 1.890 minutes (Peptide Fraction 2, PF2) and 2.484 minutes (Peptide Fraction 3, PF3). The three peptide fractions were subjected again to ACE assay to further confirm the antihypertensive activity of the chosen peptides. Among the three peptide fractions, PF2 gave the highest percent ACE inhibition (32.21), followed by PF1 (14.72) and lastly, PF3 (4.66). The percent ACE inhibition of the 24-hour protein digests is almost the same as that of PF2. This result confirmed that the antihypertensive activity of “Saba” banana is due to the peptide released from the major crude protein.

Characterization of the Peptide Amino Acid Composition Obtained from Ultra-Performance Liquid Chromatography Using Thin Layer Chromatography (TLC).

To further prove the antihypertensive activity and to determine the sequence of PF2, it was subjected to complete base hydrolysis then to thin layer chromatography (TLC). Results showed a retention factor (Rf) values of 0.42 (glutamic acid, E) and 0.13 (Lysine, K).

In Silico Analysis of the “Saba” Banana Major Protein.

“Saba” banana was found to have 501 different identities of proteins with different properties and molecular weights based from UniProtKB Protein Search. From these 501 different proteins, 49 proteins had molecular masses ranging from 19.5 kDa to 20.9 kDa. In Silico batch process of digestion for the proteins of this molecular mass range yielded five (5) protein sequences which may be the possible origins of the peptides with ACE inhibitory activity (Table 1):

Table 1. Possible Origins of the Peptides with ACE Inhibitor Activity in “Saba” Banana (*Musa balbisiana* Colla).

UniProt Entry	Name	Mass, kDa	Taxonomic Identifier
L8BSP1	Putative expressed protein	19.510	52838 [NCBI]
B0JEK1	NBS-LRR disease resistance protein	19.988	52838 [NCBI]
D7NW27	ORFIII-like polyprotein	20.369	52838 [NCBI]
D7NW44	ORFIII-like polyprotein	20.341	52838 [NCBI]
Q6Y2H5	ORFIII-like polyprotein	20.883	52838 [NCBI]

Results showed that antihypertensive peptides EK, GS, TY, FNE, FP, LKA, PT, PP, FAL and antioxidative peptides IR and VPW were found based on the sequence of these five selected proteins in "Saba" banana.

The isolated crude protein was exposed to the different digestive enzymes and results showed complete digestion of the crude protein even after 3 hours digestion time (data not shown). The ease by which the crude protein is digested over a short period of time is attributed to more cleavage sites present in the 20 kDa major crude protein (Figure 6).

The dipeptide EK which is a known antihypertensive peptide is shown to be released in all of the five protein sequences. Dipeptide EK has been found to exhibit antihypertensive activity based on the study conducted by Van Platerink *et al.* (2008).

<p>Putative Expressed Protein (19.510 kDa) M / DM / PVSEK / TS GCK / PGIQPPS PPQQAESSSP STQTR / VF / N / W / L / PTIAF / L / F / L / TY / N / SAESAY / R / SR / H / DL / PM / VAF / IV F / AY / VDL / VM / L / L / F / CL / K / QF / EK / L / S PESAPAK / R / EQ L / K / AAVW / VL / TT AL / N / L / AF / AW / R / V AEIM / PW / L / L / SV L / VW / L / M / SVSVA IGGF / Y / GL / F / IH / QGAK / DSVADG H / GY / SL / VK / N / AE L / APGEK - V</p>
<p>NBS/LRR Disease Resistance Protein (19.988 kDa) GM / GGVGK / TTL / AQQAY / N / H / ER / V K / DY / F / H / PK / VW / V CVSDN / F / N / VER / L / TK / EIIESL / T R / K / R / CDL / N / N / F / D TL / QVEVK / EK / L / TSK / R / F / L / L / VL / D DVW / N / EDSQK / W / ER / F / CAPL / R / Y / G EPGSK / IL / ITT R / Y / K / M / IAEM / VG N / PIPL / GGL / DE ASY / W / K / L / F / K / TC AF / GSEDAGEF / PH / L / EAIK / K / I AGR / L / K / GCR / W / R / QR / R</p>
<p>ORFIII/like Polyprotein (20.369 kDa) K / N / APAIF / QR / K / M / DN / CF / R / GTEK / F / IAVY / IDDIL / IF / SDSK / EAH / R / TH / L / R / QF / ITIC EEN / GL / VL / SPT K / M / K / IGVQQVD F / L / GATIGDSK / VR / L / QPH / IVK / K / VL / ETK / EESL / S ETK / AL / R / R / W / L / G IL / N / Y / AR / AY / IP DL / GK / IL / GPL / Y / SK / TSGK / GER / K / L / N / H / QDM / K / IHH / QIK / EK / VK / N / L / P EL / EVPPESI IL / IETDGCM /</p>
<p>ORFIII/like Polyprotein (20.341 kDa) K / N / APAIF / QR / K / M / DN / CF / R / GTEK / F / IAVY / IDDIL / IF / SDSK / EAH / R / TH / L / K / QF / ITIC EEN / GL / VL / SPT K / M / K / IGVQQVD F / L / GATIGDSK / VR / L / QPH / IVK / K / VL / ETK / EESL / S ETK / AL / R / R / W / L / G IL / N / Y / AR / AY / IP DL / GK / IL / GPL / Y / SK / TSGK / GER / K / L / N / H / QDM / K / IHH / QIK / EK / VK / N / L / P EL / EVPPESI IL / IETDGCM -</p>
<p>ORFIII/like Polyprotein (20.883 kDa) L / K / N / AL / AVF / QR / K / M / DDCF / R / GTE QF / IAVY / IDDI L / VF / SESK / EDH / EK / H / L / SIM / L / K / I CQK / N / GL / VL / SP TK / M / R / IAVR / EI EF / L / GAVL / GN / S K / IK / L / QL / H / IIR / K / IL / EF / SEEDL / TTR / R / GL / R / SW / L / GVL / N / Y / AR / N / Y / I PN / L / GK / L / L / GPL / Y / PK / TSPTGEK / R / M / N / APDW / K / L / IR / K / IK / EK / IK / N / L / PDL / EVPEIC Y / IIL / EVDGCM / E</p>

Figure 6. Representation of the Cleavage Sites (I) of the Five Selected Protein Sequences in "Saba" Banana.

SUMMARY AND CONCLUSION

The major protein in "Saba" banana (*Musa balbisiana Colla*) flesh was extracted using 0.125 M Tris-HCl with 50 mM NaCl at pH 7.4. The concentration of the "Saba" banana crude protein was 4.28 mg/mL. The isolated crude proteins were subjected to proteolytic digestion using pepsin, trypsin, chymotrypsin, and thermolysin. The resulting protein digests were subjected to two assays, ACE Inhibition and diphenyl-1picrylhydrazyl (DPPH) to determine antihypertensive and antioxidative activities, respectively. The 24-hour crude protein digests was found to inhibit ACE activity (36.02%). The 12-hour protein digest gave the highest values for antioxidative activity (33.14%). The 24-hour crude protein digests was subjected to Ultra-Performance Liquid Chromatography (UPLC) to determine the peptide fraction responsible for the ACE-inhibitory activity. The UPLC fraction with the highest peak (PF2) in the chromatogram exhibited an inhibitory activity of 32.21% against ACE. This fraction (PF2) was subjected to base hydrolysis. The possible sequence of the dipeptide is EK.

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