A New Banana Flavouring: Extraction and Characterization of Volatile Compounds in Cavendish Bananas (*Musa acuminata* (AAA Group) 'Cavendish')

CHBI 4990

Honours Proposal

By
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Introduction:

Banana flavouring was first formulated in the 1860s when no readily available bananas were being marketed in America. The flavouring imitated the Gros Michel (*Musa acuminata* (AAA Group)) banana, which was a readily shipped cultivar that became popular in North America a decade after the flavouring came out. By the 1960s, the Gros Michel banana became extinct due to Panama disease (a fungal blight) wiping out most of the monocultured fruit. Since then, the Cavendish (*M. acuminata* (AAA Group)) banana has become the new cultivar for mass exportation and consumption. However, with this banana's introduction, a new banana flavouring was never created and is still based on the Gros Michel banana[1].

The Cavendish banana is on the verge of extinction due to the Panama disease making a comeback[2]. This study aims to quantify and characterize the volatile compounds that coincide with flavour for the Cavendish banana, the Gros Michel banana, and artificial banana flavouring to create a new artificial banana flavouring recipe. The work done with characterizing this new banana flavour can hold historical significance due to the risk of the looming banana extinction.

Objectives:

Extraction of volatile compounds in bananas had been done using solid-phase microextraction (SPME) and simultaneous distillation-extraction (SDE) which was then separated by gas chromatography (GC) paired with different detectors[3,4]. Creating a homogenate of the banana will be done to have a guaranteed sample to examine by GC-MS, however other extraction methods will be explored to be able to find a way to extract the volatile organic compounds present that make up the flavour and odours of bananas. These methods include steam distillation, CO_2 extraction, or supercritical fluid extraction to name a few, and trying to extract volatile compounds to see if they work and how the yields compare. Some time will have to be spent optimizing the method of analysis with Gas chromatography with mass spectroscopy for proper splitting of the various volatile compounds, as well as possibly finding a way to selectively extract each volatile compound for structural analysis. From this point, the quantities of these volatile organic compounds can be quantified, and a new banana flavouring will be developed that can be attributed to the Cavendish banana.

Materials and Methods:

- CO_2 extraction:
    - CO_2 extraction used on Cavendish and Gros Michel bananas to extract volatile compounds.
    - Uses crushed dry ice, copper coil, filter paper, centrifuge tube, and an extraction vessel. May need to consider scaling up the extraction apparatus as the amount of banana to be produced may make several small-scale extractions tedious.
    - Will need to purchase dry ice, which costs approximately $5 per 2 pound of dry ice.
- Steam Distillation:
    - Used on Cavendish and Gros Michel bananas to extract volatile compounds.
    - Uses a distillation apparatus with a crushed banana and water, distillate that comes off will be the desired product
    - Will denature the banana and change the flavour.
- Supercritical fluid extraction:
    - CO_2 extraction used on Cavendish and Gros Michel bananas to extract volatile compounds.
    - Uses a supercritical fluid cell and places it under vacuum while liquid CO_2 flows through, extracting desired product
    - This technology isn't easily present at TRU, and the parts to use this extraction method are coming in but may not be ready for this project in time.

  - Will not denature the banana, however, will require a dried banana sample which may lead to denaturation, affecting flavour.

- Simultaneous distillation-extraction (SDE):
  - Can be used for extracting individual compounds from the bananas for analysis, however, it may take a long time depending on the number of compounds present, so this extraction may not be done.

- Analysis:
  - Gas Chromatography-Mass Spectrometry (GC-MS):
    - Used for analyzing and separating different compounds in the extracted oil to determine the corresponding esters that make up the flavouring.
    - Will also be used to compare and analyze with the new synthetic flavouring.
    - The use of the GC-MS will require adjustments to the carrier gas and type of column used to successfully separate the peaks with minimal to no overlap.
    - Can be used interchangeably with LC-MS, however, it is better for detecting volatile compounds, so this method is preferred.

  - Nuclear Magnetic Resonance (NMR):
    - Hydrogen NMR is used to look at different hydrogen bonding in a compound to gain structural information.

- Will be used when synthesizing banana flavouring to ensure the correct ester is created.
- It may not be beneficial to analyze extracted samples from bananas for this project if SDE is not used as analyzing a mixture of compounds gives useless results.

- Infrared Spectroscopy (IR):
  - Helpful for gaining information on functional groups present in the compound
  - Will be used when synthesizing banana flavouring to ensure the correct ester is created.
  - It may not be beneficial to analyze extracted samples from bananas for this project if SDE is not used as analyzing a mixture of compounds gives useless results.

- Olfactory methods:
  - Good ol' sniff test! Does the new synthetic banana flavouring smell like Cavendish bananas?
  - May want to get an olfactometer to find odour concentrations of compounds.

- Taste testing (assuming a food-safe lab is used with correct procedures):

        - The main way to determine if the flavouring is similar to the cavendish banana is by trying it orally.
        - In the case that the flavouring is successfully created under food-safe conditions, we will require approval from the Human Subjects Committee/Research Ethics Board to test the product.

- Statistical treatment of data:
    - Multiplets:
        - More data samples mean more precise results. Will either run samples in triplicates or more, depending on the initial results shown.
    - Standard Error:
        - For the deviation between the values within triplicates/multiplets or across identical samples.
    - Limit of Detection (LOD):
        - Lowest concentration that can be measured with significant values to be included in the report.
    - Limit of Quantification (LOQ):
        - Lowest concentration that can be measured with reasonable accuracy and precision.

- Laboratory:
    - The organic lab, organic synthesis lab, and analytical labs will be used for the duration of this study.

- Food-safe laboratory:
    - To be able to sample the synthesized banana flavouring, a food-safe lab may be required as none of the laboratories at Thompson Rivers University are food-safe. This can be outsourced to a different lab but will require travel to a nearby neighbouring city that has one that is willing to accommodate the project.

- No special equipment is needed as everything in the laboratory will suffice for this project.

Expected results:

Doing this project, I expect to be able to quantify the compounds to make a flavouring that would taste like a cavendish banana. If this synthesis is successful, it will serve as a historical marker for cavendish bananas as they are looming extinction. Regardless of the results of the new synthetic flavouring, the volatile compounds in the Cavendish banana will still be analyzed and quantified through the methods mentioned above, thus the information can be used for future work with the cultivar. The creation of a quantified banana flavouring could be used by companies to breathe new life into banana flavoured products.

Additionally, It will also be a very helpful learning experience as I plan on pursuing more food chemistry projects after completing the honours project so I can use the information I learn with this project moving forward. I have a great interest in food chemistry, so this experience will help me develop and expand my understanding. On top of this, I have no experience synthesizing and isolating volatile compounds, so the extraction process will be new to me. The method of extraction is not used much at TRU, so a method that is developed could be used moving forward in organic chemistry lab activities. Lastly, I also have no experience using the GC-MS, so using and understanding the GC-MS will be very helpful with my studies and potential future graduate studies.

Timeline:

			September				October					November				December				January					February				March				April				
		Week:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Honours	START	END																																			
Literature Review	2024-09-03	2024-10-31																																			
Procedure Development	2024-09-04	2024-10-31																																			
Extraction	2024-11-01	2024-11-30																																			
Characterization	2024-12-01	2024-12-31																																			
Synthesis of Flavour	2025-01-01	2025-01-31																																			
Create Update Presentation	2025-01-01	2025-01-26																																			
Update Presentation	**2025-01-26**	**2025-01-31**																																			
Statistic Analysis	2025-02-01	2025-02-28																																			
Writing Thesis	2025-03-01	2025-04-10																																			
Poster Conference Prep	2025-03-20	2025-03-30																																			
Poster Conference	**2025-03-25**	**2024-04-07**																																			
Thesis Defence	**2025-04-10**	**2025-04-30**																																			

Budget:

Item	Cost (in CAD)
Gros Michel Banana (8-10 lbs)	275
Cavendish Banana	5
Banana flavouring	5
Dry ice (for CO2 extraction)	30
Reagents* (see lower table)	541.85
Total	**856.85**

Reagents	Cost (in CAD)
Isoamyl acetate sample (FG)	56.15
Isoamyl butyrate sample (FG)	74.3
Isoamyl isovalerate sample (FG)	74.3
Ethyl Acetate sample (FG)	72.2
Acetic acid (100mL)	41.3
Butyric acid (100mL)	45.3
Isovaleric acid (100mL)	48.7
Isoamyl alcohol sample	47.2
Sulfuric acid (100mL)	82.4
Total	**541.85**

*Reagents are subject to change, these are high estimations

(FG) = Food Grade

The reagents above are high estimations, and a lot of the chemicals will be readily available by the chemistry department so the estimated cost will be lower than stated. The expenses above for this Honours project will be covered by U-REAP grant funding. If U-REAP funding for this project is not secured, then the project will be covered by the supervisor's grants as a back-up.

Literature sources

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(2) "Bananas: The Uncertain Future of a Favorite Fruit." *NPR*, 2011. https://www.npr.org/2011/08/30/139787380/bananas-the-uncertain-future-of-a-favorite-fruit (accessed May 7, 2024).

(3) Pino, J. A.; Febles, Y. Odour-active compounds in banana fruit cv. Giant Cavendish. *Food Chemistry* **2013**, *141* (2), 795–801. https://doi.org/10.1016/j.foodchem.2013.03.064.

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