Cole Rohrbaugh

Lipid Extraction from Bee Pollen for Medicinal Benefit

Introduction and Background

Lipids are a type of fat found all throughout nature, and they serve many different functions depending on the lipid. There are two different lipids: good lipids which can help humans, and bad lipids which negatively affect humans. Good lipids help with metabolism; thus, they are responsible for burning calories. Bad lipids are unsaturated fats which affect the body negatively. Additional functions of lipids are metabolic processes like signaling, secretion, and energy storage.[1] While not much research is done in relation to lipids, it is known they are possible causes for different diseases like Alzheimer's, diabetes, and cancer.[1] In spite of knowing these diseases have potential connections to lipids, the unknown of lipids is a vast world to be explored for medicinal reasons.

Lipid extraction is a huge area of research as many different researchers are trying to discover new ways of quickly extracting lipids. In order to understand the different properties and functions of lipids, the lipids must first be extracted normally from tissue.[2] Extracting the lipid allows for an extensive analysis of a lipid allowing much more to be learned about the lipid.

Complex methods of extracting lipids can be eliminated using new way of using superabsorbent polymers (SAPs) to perform the extraction. This method reduces the time to perform an extraction (5-10 min total(?) or is this the reduced amount of time taken off the top?), allowing the sample to be ready quicker. This method is better at dealing with small samples; conventional methods were not ideal for small size samples.[1] The extraction is done well, and this method did not hurt determination of lipids. It appears to be a very reliable and reproducible method to be used in the future.

Deleted: Possible

Deleted: s

Deleted: contain

Deleted: today

Commented [JMF1]: Reference?

Deleted: and efficiently

Commented [JMF2]: Are you referring to another study where it "is" done well? Do you want to say something like "When the extraction is done correctly, it allows for the determination of lipids in the sample" ??

Other applications that lipid extraction could be useful for is in the field of archaeology. Over many years archaeologists have tried to discover as much as they can related to humans' lifestyles throughout history. They have had to rely on chemists to test different samples of what they found by performing different types of extractions, but this article talks about how one extraction method is found to be better than the other. Supercritical fluid extraction (SFE) is a new type of extraction that analytical chemists use quite often because it is a method that is found to be superior to other types of extractions.[3] compared 12 different samples using the SFE method they created. It was discovered that SFE yielded a better result in terms of quantity and time than other methods. The SFE was much more efficient and it is less hazardous as it requires ethanol and water which makes it less toxic to the environment. Overall, it was found the SFE was more efficient and safer; thus, it is a better method to use when attempting to extract lipids.[3]

In this work, we propose a method for the lipid extractions of bee pollen from three plants (crocus, hyacinth, foxgloves) with the goal of characterizing the lipids extracted with a new method using the SFE/SFC and MALDI-TOF instrumentation, and the SAPs lipid extraction method. The goal of characterizing the lipids extracted is to find medicinal benefits from the lipids extracted.

Significance

By being able to extract lipids from bee pollen, it could open up new possibilities in the medicinal world. The lipid extraction from bee pollen could possibly become a cheap option in developing anti-inflammatory medicines as there is always bee pollen being made by bees[4]. This research can help determine how efficient it will be to extract lipids from three different plants, and a combination of plants, with the ultimate goal of creating a method to efficiently extract lipids.

Commented [JMF3]: You haven't really mentioned applications before this, so it can't be "other".

Deleted: about the past

Commented [JMF4]: What article? You need to reference it, or it's authors here.

Commented [JMF5]: This seems out of place. Mistake?

Commented [JMF6]: Again, what study, and what is the reference?

Commented [JMF7]: Was/is: decide what tense you want to use

Also, this is again referring to a paper that has not been cited.

Each sentence needs to be cited if you are taking info from a source.

Deleted: W

Formatted: Not Superscript/ Subscript

Commented [JMF8]: Spell out your acronym before using it

Commented [JMF9]: How will you be finding medicinal benefits? (I don't think you will be…)

Suggested: By extracting lipids with the SFE the goal will be to improve on the extraction and characterization such that a potential medicinal benefit of bee pollen lipids may be found.

Commented [JMF10]: This statement is a little awkward. Why is the continuous production of pollen related to inflammation? (it's not) Think about what you are trying to communicate here

Research Design

Collecting bee pollen will be done by scraping the pollen mixed with nectar from the three plants mentioned earlier. For this research, new molecules/compounds are not being designed, but the only necessary design is designing the pollen extraction.

Methods

SAPs Experimental Method

Superabsorbent polymers (SAPs) are quite useful in the process of extracting lipids from a known source containing lipids; in fact, it was found to have a 95-100% recovery rate.[1] For this research, extraction of lipids from each type of bee pollen will have three trials, so a total of nine trials can be completed using this method. In order to be effective in the extraction, the first step would be to dissolve the bee pollen in an organic solvent like methanol or chloroform with a total volume of 10 mL. Dissolving the bee pollen is a crucial step in making sure this method would be effective, and there will be nine samples being dissolved in the organic solvent. Before the dissolved pollen can be used with the SAPs, it first must be dried and then re diluted.[1] After the pollen would be diluted again with methanol, distilled water would also be used as a part of the dilution with a total of 10 mL of distilled water used per sample.

To perform the lipid extraction, around 20 mg of SAPs will be used per trial as the extraction process. For each trial, around 100 microliters of a sample will be used. The samples will be dropped onto the SAPs powder in order for the SAPs to extract the lipid. Once the samples are dropped onto the SAPs, it will sit there for a minute to generate a gel. From the gel, lipids would be extracted from the gel using a pipet.[1] From all of this, the extracted samples will then be run through more instrumentation with the ultimate goal of characterizing the lipids

Deleted: hopefully

Deleted: in the paper

Deleted: Learning how to collect the pollen must be done in order to have enough bee pollen to perform experimentation

Commented [JMF11]: Your design needs work. The design includes what you are collecting on the instrumentation. What data will you collect? You need to mention the SFE, etc. Samples will be extracted by the SFE and then what? What will you analyze or characterize? The design should spell that out.

The methods are the exact "things and amounts" you will be doing and using.

Formatted: Superscript

Commented [JMF12]: This is your design, basically, except you don't need times and amounts of solvent.

Then, your method would be "Bee pollen will be dissolved in 10 mL of XX solvent after extraction. Pollen will be diluted to XX, with a total of XX samples". Something like that for METHOD

Commented [JMF13]: Pipette?

Commented [JMF14]: This needs to be worked out.

found in the bee pollen. Also, it will be determined whether this method is efficient in the extraction of lipids from bee pollen.

SFE Experimental Method

The use of supercritical fluid extraction has been done before in reference to lipid extraction, but this experiment will focus on extracting lipid from bee pollen. The parameters (oven temperature and pressure) for lipid extraction based on the past experiment determined what the vital temperature and pressure should be for extraction.[3] For temperature, it was determined the oven should be set to 50℃, and the pressure in the system should be kept at 500 bar.[3] For this research, the supercritical gas to be used should be CO_2. Additionally, the co-solvent planned to be used in this experiment is methanol as the collection flask should have methanol in it, so the supercritical fluid will contain lipid and methanol. The perfect extraction time according to Deviese and others was 90 minutes, as this was the perfect amount of time to extract all of the lipids in a sample.[3] For the said research, the bee pollen will be packed into a vessel with glass wool at the said temperature and pressure and be run for 90 minutes. There is no extra preparation the bee pollen must go through as it will just be placed right into the vessel. For each type of plant, there will be 8 trials that will be run through the SFE, giving a total of 24 trials to be run through the SFE. For each trial, there will be 3.000 grams of bee pollen used.

Characterization of Lipids

In order to characterize the lipids found by the extraction methods said above, they must be run through different instrumentation. To start with, every sample would be run through the high-performance liquid chromatography (HPLC) with the goal of identifying lipids based on retention times and peaks. A study determined a good way to run the lipids through the HPLC with the different parameters that must be decided before being run through. First, the study

Commented [JMF15]: Right here your text changes to gray. Change all of it back to black please

Deleted: determined

Commented [JMF16]: Again, this is more of your design.

The methods should be like the methods section of a journal article, just in future tense. (2.0 grams of A will be combined with 3.0 grams of B (20 moles of each) in solvent Y with stirring for 17 hours)

Commented [JMF17]: See my comments above out design and methods. We can talk about this too if you need some guidance

determined the flow rate to be used was 4 mL/min which worked, and it was determined to have the column temperature to be 40℃.[5] Using the HPLC will be one way to attempt to characterize lipids from extraction.

Another method that will be used to characterize the lipids is the MALDI-TOF. A study which used the MALDI-TOF to analyze lipids will influence the method used for this research. In terms of the lipid extract analysis, the light intensity will be set from 55-60% to do the characterization.[6] Each trial from each method will be run through the MALDI-TOF. From the spectra given from the MADLI, it will be determined what lipids are present in the pollen.

Commented [JMF18]: Data? The Maldi basically gives you a M/Z ratio with respect to abundance in your sample. It's not really spectroscopy (where you get a spectrum). It's spectrometry…that's slightly different.

For both of the said characterization methods there will be standards that will be tested. The standards will allow the unknown lipids from bee pollen to be characterized easier as the different tests from standards can be compared to the unknowns in an attempt to characterize the lipid. The lipid standards that will be used are phosphatidylcholine, triglyceride, and a fatty acid.

Materials and Budget

Material	**Amount Needed**	**Cost**	**Company**
Crocus Plant	10 bulbs	$12.95	Eden Brothers
Hyacinth Plant	50 bulbs	$9.95	Breck's
Foxgloves Plant	1 plant	$7.98	American Meadows
Methanol	1 Liter	$45.00	Sigma-Aldrich
Pollen Mix	1 pound	$12.99	Thrive Market
SAPs	1 pound	$19.99	Amazon
Phosphatidylcholine	100 mg	$48.00	Sigma-Aldrich
Triglyceride	1 AMP	$35.25	Sigma-Aldrich
Fatty Acid (Stearic Acid)	500 mg	$25.75	Sigma-Aldrich

Timeline of Proposed Work

Time Needed(Hours)	40 hours	40 hours	20 hours	20 hours
Activity	SFE Extraction per plant, and mix	SAPs Extraction per trial	HPLC for every trial	MALDI-TOF

Commented [JMF19]: My guess is that this will take longer

How many credits do you plan to sign up for in the fall? It will be helpful to put that here in this table

Deleted: ¶

Deleted: ¶

Formatted: Indent: Left: 0"

Formatted: Font: Bold

References

1.) Bang, G.; Kim, Y.H.; et al. On-Chip Lipid Extraction Using Superabsorbent Polymers for Mass Spectrometry. *Analytical Chemistry.* **2017,** *89(24),* 13365-13373.

2.) Khoomrung, S.; Chumnanpuen, P.; et al. Rapid Quantification of Yeast Lipid using Microwave-Assisted Total Lipid Extraction and HPLC-CAD. *Analytical Chemistry.* **2013,** *85(10),* 4912-4919.

3.) Deviese, T.; et al. Supercritical Fluids for Higher Extraction Yields of Lipids from Archeological Ceramics. *Analytical Chemistry* **2018,** A-E.

4.) Li, Q.; Liang, X.; Zhao, L.; Zhang, Z.; Xue, X.; Wang, K.; Wu, L. UPLC-Q-Exactive Orbitrap/MS-Based Lipidomics Approach To Characterize Lipid Extracts from Bee Pollen and Their in Vitro Anti-Inflammatory Properties. *Journal of Agricultural and Food Chemistry.* **2017**, *65*(32), 6848–6860.

5.) Willmann, J.; Mahlstedt, K.; Leibfritz, D.; Spraul, M.; Thiele, H. Characterization of Sphingomyelins in Lipid Extracts Using a HPLC-MS-Offline-NMR Method. *Analytical Chemistry.* **2007**, *79*(11), 4188–4191.

6.) Vitale, R., Angelini, R., Lobasso, S., Capitanio, G., Ludwig, B., and Corcelli, A. (2015) MALDI-TOF MS Lipid Profiles of Cytochrome c Oxidases: Cardiolipin Is Not an Essential Component of the Paracoccus denitrificans Oxidase. *Biochemistry. 54*, 1144–1150.

Commented [JMF20]: Your font here is again in gray (and below). Check that it is all black