



Lipid Extraction from Bee Pollen for Possible Medicinal Benefits

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Introduction

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 negatively affect the body. Additional functions of lipids are metabolic processes like signaling, secretion, and energy storage. 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. Supercritical fluid extraction (SFE)¹ is a new type of extraction that analytical chemists use quite often as it is found superior to other types of extractions. One study discovered that SFE yielded a better result in terms of quantity and time than other methods. The SFE appears to be much more efficient and less hazardous to the environment as it requires ethanol (or methanol) and water. we propose a method for the lipid extractions of bee pollen² from two different pollens from two different farms with the goal of characterizing the lipids extracted with a new method using the SFE, and high-performance liquid chromatography (HPLC).



Figure 1. Supercritical fluid extraction instrument

Figure 2. Bee pollen

Objective

- Determine the efficiency of the super critical fluid extraction method by using triglyceride mix and stearic acid as standards for the HPLC to compare against the different bee pollen extracts.
- Determine the optimal conditions for extraction by varying the temperature, pressure, and extraction rate.
- Determine whether any lipids are found to be present and their respective concentration.

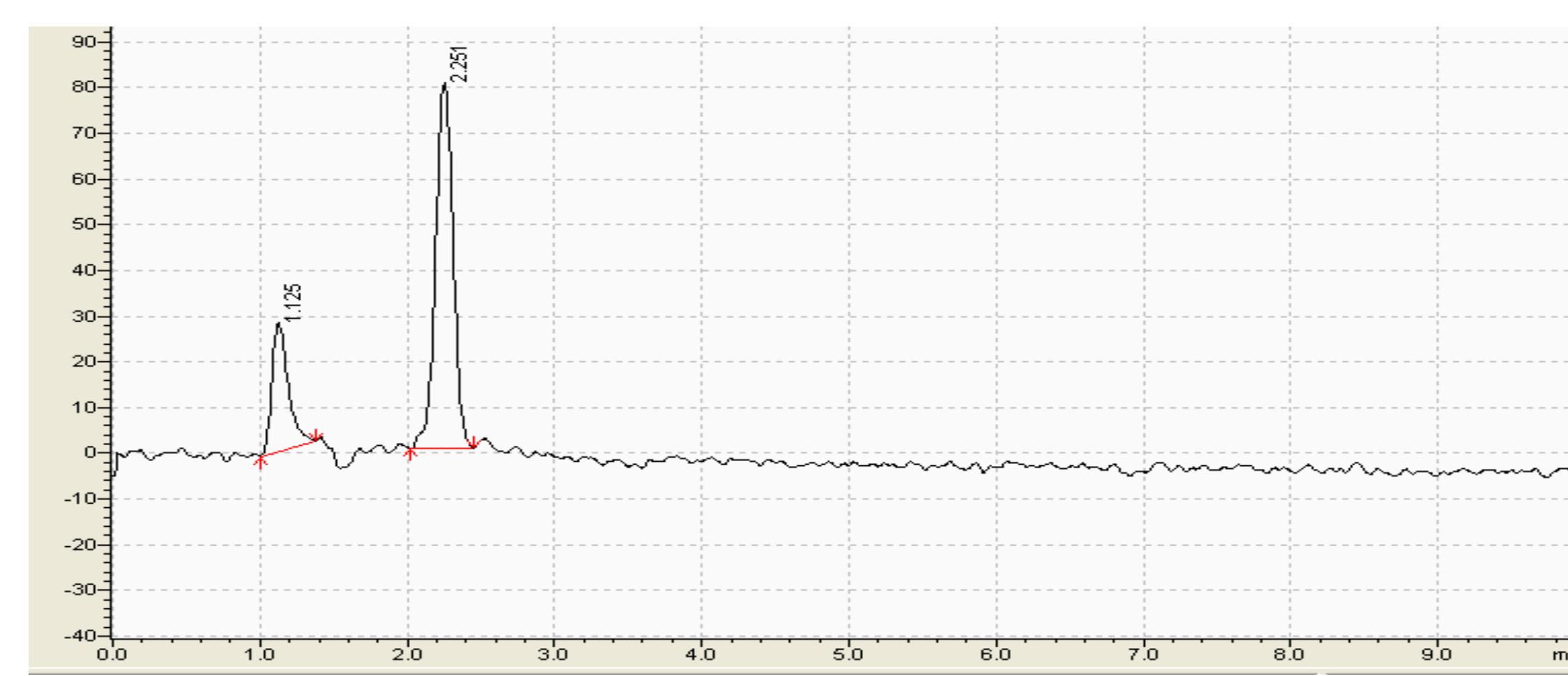


Figure 3. Spectra of a 1000ppm standard triglyceride mix

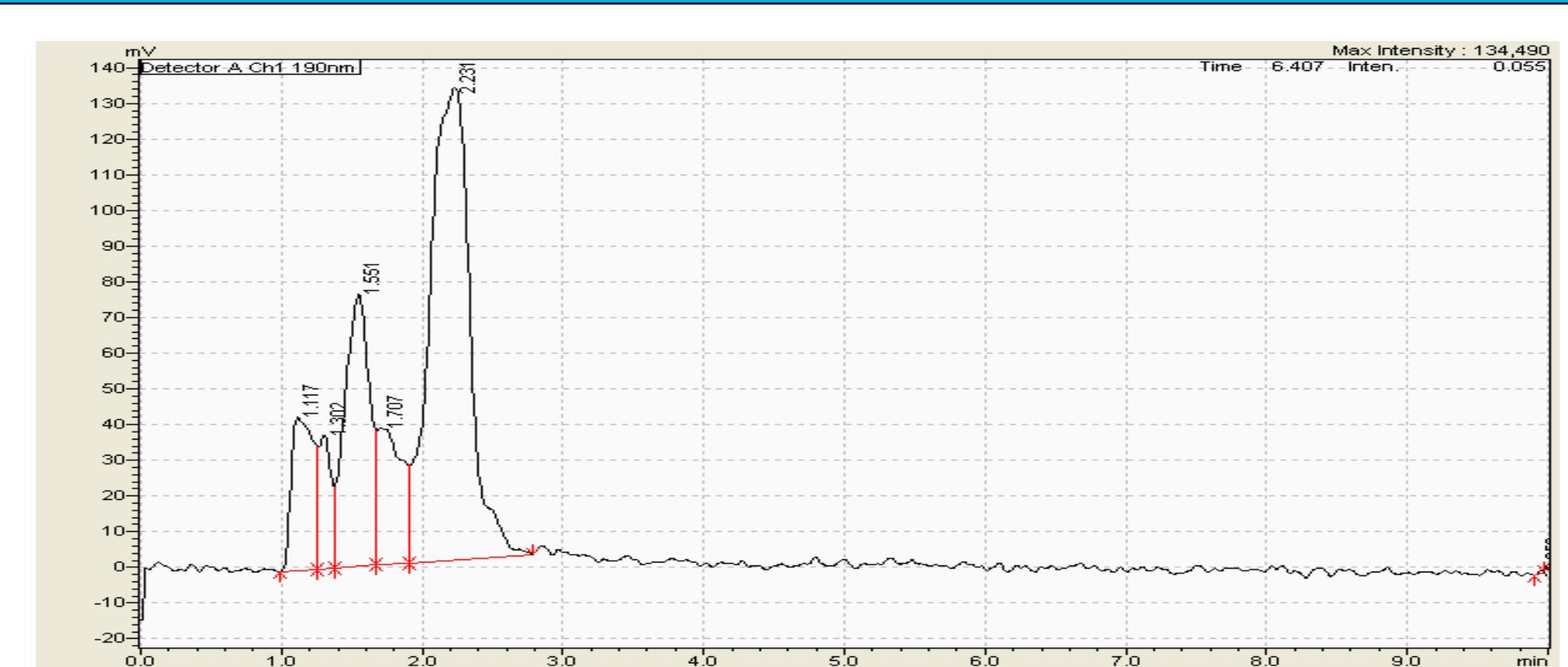


Figure 4. Spectra of a bee pollen sample at 45 degrees Celsius

Methods

- For SFE, pressure (200 or 300 bar), temperature (45 °C or 60 °C), and extraction times (15 or 30 minutes) were conditions for extraction
- Two different bee pollens were run through for each set of parameters and collected in methanol twice
- Standards were created using a serial dilution to make 1000, 500, 250, 100, and 50 ppm in order to preserve chemical use.
- For the HPLC, the oven was set at 40 degrees Celsius and the flow rate was set to 1 ml/min
- Data was observed from the chromatography allowing a calibration curve to be created to determine lipid concentration in extract

Results

Bee Pollen Type	Pressure	Temperature	Time	sample #	Concentration of triglyceride
NR	300	45	30	1	6838.837468
NR	300	45	30	2	12206.4328

Table 1. Table of the non refrigerated bee pollen extracts with lipid confirmation.

Bee Pollen Type	Pressure	Temperature	Time	sample #	Concentration of stearic acid
RF	300	60	15	1	610.0879212
RF	300	60	15	2	13.10877371
RF	300	60	30	1	90.27227951
RF	300	45	15	2	198.9401123

Table 2. Table of refrigerated bee pollen extracts with lipid confirmation.

Results (Continued)

$$\text{percent of lipid confirmation} = \frac{\text{samples confirmed}}{\text{total number of extracts}} \times 100$$

$$\text{percent of lipid confirmation} = \frac{6}{21} \times 100 = 28.5\%$$

- Out of the 21 samples, 2 samples were found to have triglyceride and 4 had stearic acid
- Success rate of 28.5% in lipid extraction

Discussion

- It can be confirmed lipids were extracted from bee pollen using the SFE. It can also be confirmed both triglyceride and stearic acid appear to be present in some of the different bee pollen extracts.
- Figure 3 shows a standard triglyceride mix spectra, and figure 4 shows an example of a bee pollen extract spectra. Figure 4 shows the confirmation of some lipids present; however, it shows that not every peak found was one of the standard lipids to compare against.
- Tables 1 and 2 show non-refrigerated bee pollen appears to contain triglyceride and not stearic acid while refrigerated bee pollen contains stearic acid but not triglyceride respectively.
- Overall, it appears 300 bar was the optimal pressure condition, 30 minutes of extraction time was the optimal extraction time, and it appears 45 degrees Celsius was the optimal temperature as it produced the highest concentrated lipids.

Conclusion

- With the findings in this experiment, it could be determined bee pollen might be a source lipids are extracted from to create anti-inflammatory medicine one day as 28% of the extracts contained lipids.
- A future study could include higher pressures and temperatures to see the difference in those; as well as, using a second instrument in an attempt to characterize the extract from the SFE.
- A limitation in this study is the low amount of standards as the researcher would have used more if standards had been affordable.
- A second limitation is the inconsistency of the SFE as running the instrument was quite difficult leading to poor samples.

References

- Bang, G.; Kim, Y.H.; et al. On-Chip Lipid Extraction Using Superabsorbent Polymers for Mass Spectrometry. *Analytical Chemistry*. 2017, 89(24), 13365-13373.
- Deviese, T.; et al. Supercritical Fluids for Higher Extraction Yields of Lipids from Archeological Ceramics. *Analytical Chemistry* 2018, A-E.
- 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.
- 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.

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