# **Characterization and Analysis of Curcumin Extracted from Turmeric using Mass Spectrometry and UV-Vis Spectroscopy** Cecilia Russo and Anna M. Fedor

### Abstract

Turmeric is a spice most commonly found in Indian cuisine that is known for its medicinal properties. The most active compound that can be found in turmeric is curcumin. Curcumin is a polyphenol that is best known for its antioxidant and anticancer properties. Curcumin is mostly insoluble in water so the extraction of curcumin from turmeric was achieved by using ethanol or dichloromethane/hexane as the solvent. Since turmeric is not soluble in these solvents, a small amount of turmeric was soaked in larger amount of ethanol or dichloromethane/hexane to allow the curcumin to be dissolved out. The resulting solution can be filtered to separate the dissolved curcumin from the solid turmeric. Computational analysis can be done using instruments such as a UV-Vis and Mass Spectrometry to confirm whether or not the extraction process was a success. One of the reasons that curcumin is not widely used to treat carcinomas is due to it poor absorbance and digestion by the human body. The effects of the stomach enzymes pepsin and a-amylase on the digestion of curcumin is one of the main focuses of this research.

# **Characteristics and Digestion of Curcumin**

Curcumin is known for its anti-inflammatory, anti-cancer, and antioxidant properties which is get from the two hydroxyl groups that are great at neutralizing free radicals.

Curcumin had low bioavailability with per os ingestions due to its instability at low pH (such as that of the stomach) and subsequently is quickly metabolized and secreted before it could be absorbed by the body.

The metabolism of curcumin takes place primarily in the liver but also in the kidney and intestines. The only way to properly absorb curcumin into the bloodstream is by per os ingestion which is why finding a way to bypass the stomach acid for better metabolism of curcumin is difficult.



### **Curcumin Extraction Methods**

### Extraction method 1: ethanol solvent







## **Curcumin Interaction with Pepsin**

Pepsin is a stomach enzyme that aids in digestion. Previous studies (Ying, et al, 2015) using UV-Vis spectroscopy suggest that curcumin can bind with pepsin via hydrogen bonding.

A strong peak is observed at 194 nm (pepsin). With the slow addition of curcumin the absorption peak red-shifts due to structural changes that pepsin undergoes when binding with curcumin.

A pepsin-cucumin complex forms resulting in an absorption peak 274 nm. There is also enhanced absorbance with each curcumin addition which shows that curcumin does bind with pepsin.





Figure 2: Structure of pepsin ( and a-amvlase (right)

# **Curcumin Interaction with a-Amylase**

a-Amylase is a pancreatic enzyme that has been shown to bind to the polyphenolic compounds found in green tea and cinnamon. Given curcumin's similarity to these polyphenols, it was hypothesized that a-amylase could also bind to curcumin. This could be done by forming an a-amylase-curcumin complex similar to the pepsin-curcumin complex found by previous researchers.





Figure 3 was obtained using a small amount of solid from extraction method 2. Curcumin is known to have an absorption peak at 424 nm which matches perfectly with the results in Figure 3. This is sufficient evidence to say that curcumin was successfully extracted from turmeric.

Figure 4 was obtained by dissolving curcumin and a-amylase separately in a phosphate buffer and combined them to determine if a a-amylase-curcumin complex is formed.

As seen in Figure 4, the peak at 277 nm represents the a-amylase. There is no peak observed at 424 nm which indicates that the curcumin was not able to dissolve in the phosphate buffer. There was no observed red-shift or complex formed.

# Department of Chemistry and Biochemistry, Misericordia University, 301 Lake Street Dallas, PA 18612

Figure 4: Absorption Spectrum for Curcumin-Amylase Complex

## **<u>Characterization of Curcumin Using Mass Spectrometry</u>**

Figure 5 was obtained using Atmospheric Solids Analysis Probe Mass Spectrometry (ASAP-MS). This allows for analysis of solid samples by determining its molecular weight. All other peaks on the spectrum result from fragmentation of the molecule or impurities. The molecular weight of curcumin is known to be 368.5 g. The strong peak at 367 m/z was identified as curcumin. It was concluded that curcumin was successfully extracted from turmeric using extraction method 2.



Extraction method 1 was not effective. The product was too hard to work with, making it difficult to run analysis.

Extraction method 2 was very successful. This method produced a solid that was easy to work with which was characterized using ASAP-MS.

Dissolving curcumin in a phosphate buffer in order to initiate an interaction with a-amylase was unsuccessful but does not eliminate the possibility of a a-amylase-curcumin complex forming.

Increase the solubility of curcumin and measure the red-shift of the a-amylase-curcumin complex using UV-Vis spectroscopy. Examine the possible interactions between pepsin and curcumin. Investigate metabolism of curcumin in low pH (stomach acid). Improve the extraction methods used in this study.

Kyra Grzymski and Shelby Burke Misericordia University Department of Chemistry and Biochemistry

Anderson, A.M.; Mitchell, M.S.; Mohan, R.S. Isolation of Curcumin from Turmeric. J. Chem. Ed. 2000, 77, 359-360. Curcumin. Linus Pauling Institute: Micronutrient Information Center. https://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/curcumin. (accessed September 25, 2019). Heffernan, C.; Ukrainczyk, M.; Gamidi, R. K.; Hodnett, B. K.; Rasmuson, A. C. Extraction and Purification of Curcuminoids from Crude Curcumin by a Combination of Crystallization and Chromatography. Org. Process Res. Dev. 2017, 21, 821-826. Phosphate Buffer (pH 5.8 to 7.4) recipe and preparation. AAT Bioquest. https://www.aatbio.com/resources/buffer-preparations-and-recipes/phosphate-buffer-ph-5-8-to-7-4. (accessed October 30, 2019). Plate Express. Advion. https://www.advion.com/products/plate-express/. (accessed November 4, 2019). Ukrainczyk, M.; Hodnett, B. K.; Rasmuson, A. C. Process Parameters in the Purification of Curcumin by Cooling Crystallization. Org. Process Res. Dev. 2016, 20, 1593-1602. Ying, M.; Huang, F.; Ye, H.; Xu, H.; Shen, L.; Huan, T.; Huang, S.; Xie, J.; Tian, S.; Hu, Z.; He, Z.; Lu, J.; Zhou, K. Study on interaction between curcumin and pepsin by spectroscopic and docking methods. Int. J. Biol. Macromol. 2015, 79, 201-208.

## Conclusions

## **Future Work**

## Acknowledgements

## References