Extraction of Curcumin from Turmeric Efficiently

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Abstract

Cystic Fibrosis is a one of the most deadly diseases in the world. The research done by the researchers at Yale found out that Curcumin, a major constitute of Turmeric, treats Cystic Fibrosis. The process for extracting curcumin efficiently consists of suction-filtration, TLC, reflux for one hour, and the use of the chemicals dichloromethane and hexanes. 20g of Turmeric yielded approximately 5g of Curcumin. However, can curcumin be extracted efficiently in a microwave? A reflux apparatus with a two flasks, one which is opened one side and one which is open on two sides. A hole was made in a rubber stopper in which a test tube is inserted. Curcumin dissolved in Dichloromethane will be inserted into the bottom flask then put in the microwave. To set up a reflux the top flask will be filled with ice. The ice will surround the test tube and condense the vapors in it. Because there is an increase in pressure in the test tube, a small hole was made in the test tube. To avoid excessive heating of the rubber stopper and boiling of the dichloromethane, the reflux apparatus is only put in the microwave for about 15 seconds, then removed for 5 seconds.

Introduction

What is Curcumin?

Curcumin is an active ingredient in turmeric, a spice mainly used in South Indian and Thai cooking. Curcumin is 1.5-2.0% by weight of the turmeric root (Anderson et. al., 2000). Curcumin is a yellow-orange substance that makes turmeric yellow and mustard yellow. See Figure 1.

Figure 1.
www.elsiglodetorreon.com.mx
The Figure above shows the orange-yellow color of turmeric

Curcumin is a 7- Carbon chain connected by an aromatic ring on either side. There are two parahydroxl groups; the two hydroxides are parallel to each other. There are two keto groups which is a double bond between carbon and oxygen, and two double bonds in the chemical structure of curcumin (Anderson et. al., 2000). See Figure 2.

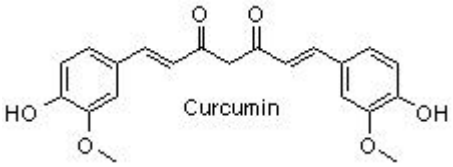

Figure 2
www.chemicalforums.com

Curcumin is an antioxidant, anti-inflammatory, anticancer supplement. It has a low level of toxicity so humans can consume it without any side effects. However if curcumin is consumed in large doses it can cause the calcium level in the cells to decrease, because curcumin is known to block the SERCA pump that allows calcium to enter the cell.

Curcumin and Cystic Fibrosis

In recent studies, researchers at the Yale School of Medicine found that curcumin helps treat Cystic Fibrosis, a major genetic disease that affects many Caucasians young or old across the world (Egan et. al., 2004).

Cystic Fibrosis is a genetic disease that mainly affects the lungs and the gut. In a healthy, fit person mucus flows over air passages in the lungs to remove any bacteria or debris. This bacteria and debris are released when one sneezes or spits out saliva. However, people that have Cystic Fibrosis have mucus that is sticky and thick causing it to remain in one spot. The mucus is a very stable environment for bacteria and thus a bacterial colony forms in the mucus along the passage-ways in the lung. Many people with Cystic Fibrosis die from the bacteria that form in the mucus.

There are many symptoms of Cystic Fibrosis. They would be: appears out of breath, has a persistent cough with thick mucus, has salty sweat, stunted growth, may become easily dehydrated, may lose weight because of the inability to absorb nutrients, and more (Rowe et. al., 2005). Other diseases that people get because of having Cystic Fibrosis are: liver disease, diabetes, inflammation of pancreas, and gallstones.

Right now there are many temporary treatments for Cystic Fibrosis. They include a healthy diet, consumption of proteins and vitamins, decongestants (open up the air way

to let the mucus drain out), anti-inflammatory drugs, pancreatic enzymes which help with digestion, chest or back clapping, antibiotics, and gene therapy (Rowe et. al., 2005).

During gene therapy a normal copy of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) DNA is delivered to the lungs as an aerosol. This aerosol then has to get to all the cells with the mutation, but sometimes it is not successful (McCray, 2001).

The research in Cystic Fibrosis is progressing every year. As technology advances there will be more and more treatments for Cystic Fibrosis and a cure might be discovered.

Cause of Cystic Fibrosis

Cystic Fibrosis is caused by a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). The CFTR gene encodes a protein however, with a mutation the protein does not form correctly. The protein gets misfolded and then cannot function. CFTR gene is found on Chromosome 7 on the "q" arm of the human chromosome (DEPERP, 2003). See Figure 3.

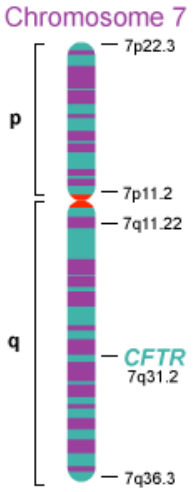

Figure 3
www.ornl.gov
The CFTR gene is located on Chromosome 7.

Cystic Fibrosis is an autosomal recessive disorder. An autosomal recessive inheritance means that the gene has to be on one of the chromosomes and there has to be two copies of the gene. For a person to have Cystic Fibrosis they must have two copies of the mutated gene CFTR. If a person has a normal CFTR and a mutated CFTR that person would not get Cystic Fibrosis, but that person would be a carrier of the disease, and can pass it down to their offspring. If both parents are carriers there is a 25% chance that one child will have Cystic Fibrosis and a 50% chance that the child will be a carrier. A carrier of Cystic Fibrosis has to be paired with another carrier for the offspring to get Cystic Fibrosis. See figures 4 and 5.

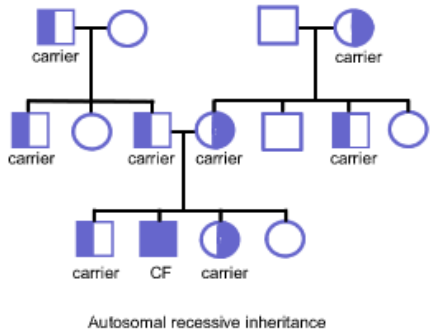

Figure 4
www.cysticfibrosismedicine.com

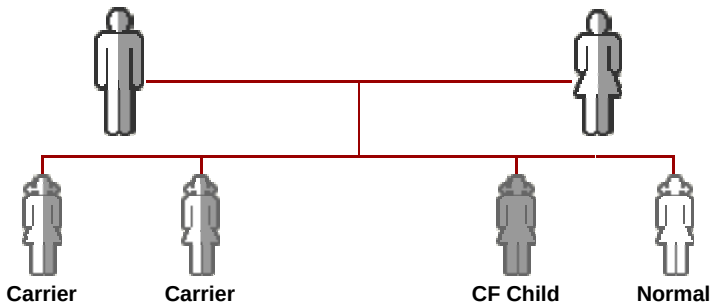

Figure 5
www.cfnz.org

The CFTR gene forms a protein that acts as a chloride channel on the plasma membrane. The CFTR protein consists of 1480 amino acids see Figure 6 (DEPERP, 2003). The chloride channel actively transports chloride ions across the cell membrane using ATP energy (DEPERP, 2003).

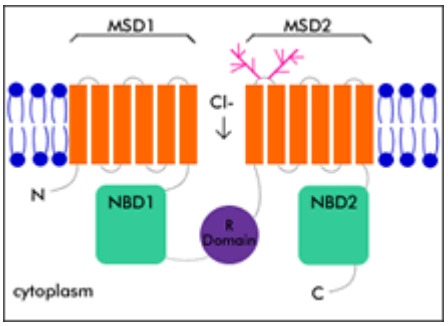

Figure 6
www.ornl.gov

This chloride channel transfers the chloride ions across the cell membranes in the lungs, pancreas, liver, and skin. If the chloride channel does not get integrated into the cell membrane the chloride ions cannot pass across the membrane. This upsets the chloride and sodium ion balance causing the mucus that is sent over the air passages to be thick and sticky.

In the endoplasmic reticulum (ER) the CFTR protein gets misprocessed because of the mutation ΔF508 in the CFTR gene (Egan et. al., 2004). Instead of going to the membrane and creating a chloride channel, a chaperone protein takes it and diminishes it. A chaperone protein is a protein that helps other proteins with their folding. Because there is no way for the chloride ions to leave the cell, there is an unbalance of chloride

and sodium ions. The researchers at the Yale School of Medicine found out that chaperone proteins depend on high calcium levels in the cytoplasm. Curcumin is known for reducing the level of calcium in the endoplasmic reticulum. Curcumin lowers the calcium level in cell because it blocks the Sarcoplasmic Endoplasmic Reticulum Calcium Pump (SERCA) (Egan et. al., 2004). The SERCA pump allows calcium to enter the endoplasmic reticulum by expelling energy (ATP). When curcumin is added in the cell, it blocks the SERCA pump allowing the chaperone protein to release the ΔF508 CFTR protein. The ΔF508 CFTR protein then travels to the plasma membrane and forms a chloride pump.

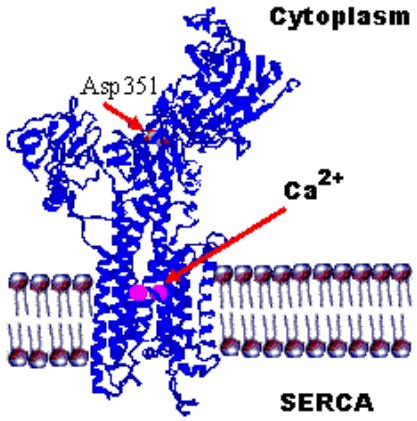

Figure 7
www.cellscience.com

When curcumin was inserted into mice with the ΔF508 mutation the mice lived for several weeks. Mice with Cystic Fibrosis only live for about four weeks (Egan et. al., 2004). This was a great accomplishment for the researchers at Yale.

Curcumin is still not an official treatment for Cystic Fibrosis because the side-effects are for large amounts of curcumin in the human body is unknown. Researchers now have to decide the safe ratio of the amount of curcumin to the weight of the person.

Doctors are urging their Cystic Fibrosis patients to not use curcumin until it is safe to do so because the FDA has not approved of using turmeric to treat Cystic Fibrosis yet. The mice that were tested at Yale were given 45mg of curcumin per kilogram of body weight and that was safe for the mice. However, this ratio will not work for humans because humans have a different characteristics and DNA. The next step for researchers is to find the right ratio of curcumin to weight and find out if that is going to do any harm for patients with Cystic Fibrosis.

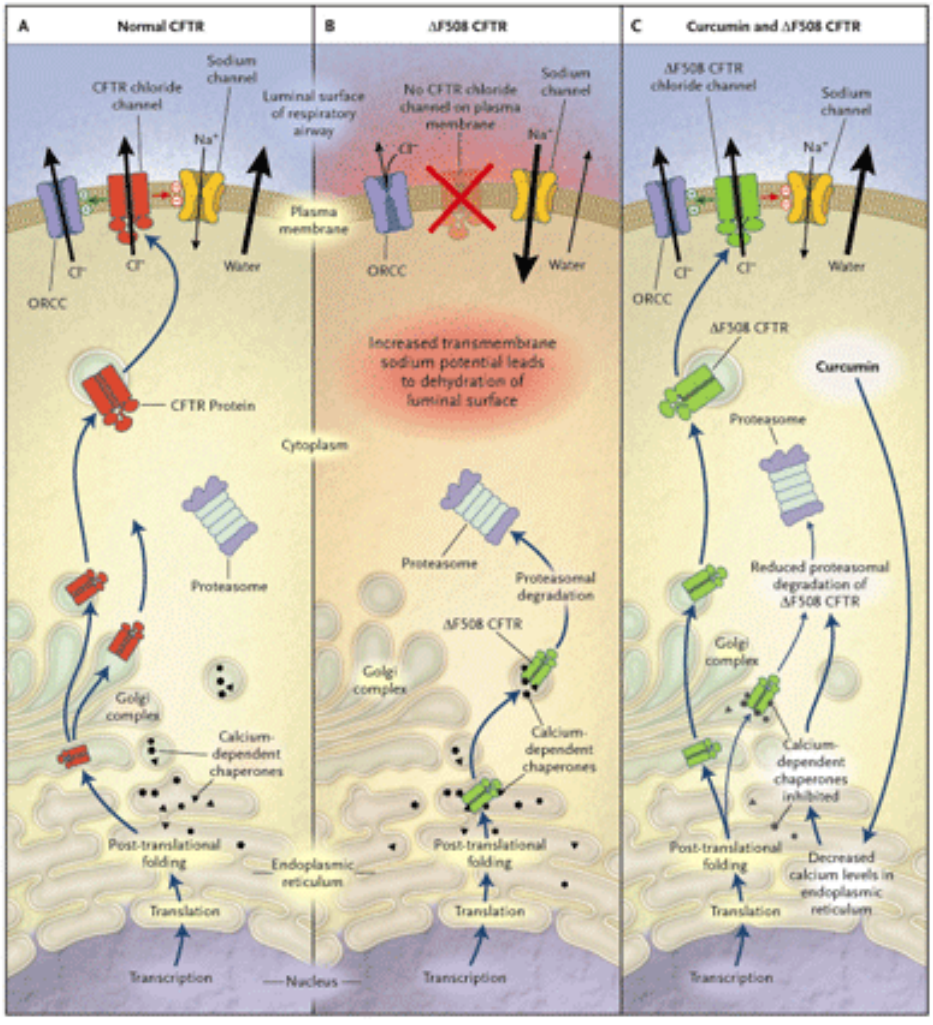

Figure 8
www.nejm.org

With a normal CFTR protein it lets the chloride ions move across the gradient, however with a ΔF508 CFTR protein there is no chloride channel that lets the chloride ions move across the gradient. When curcumin is inserted the calcium level reduces causing the chaperone proteins to not function and frees the CFTR protein. Now the chloride ions can freely move across the gradient.

Isolation of Curcumin

As researchers are finding more ways of using curcumin to treat other medical diseases, it has to be isolated from turmeric. Curcumin can be found in pharmacy stores, that curcumin has been isolated from turmeric in labs. The chemicals needed to isolate this curcumin are dichloromethane, hexane, and methanol (Anderson et. al., 2000). This process involves trituration, using a reflux apparatus, suction-filtering, TLC, and chromatography. I will be trying to isolate curcumin efficiently by creating a reflux apparatus for the microwave.

Materials

Chemicals/Consumables	***Supplies***	***Equipment***
50 ml Dichloromethane 20g Turmeric 20mL Hexanes .3 mL Methanol	Two flasks: polypropylene and glass Test tube Gloves Safety glasses	Microwave Suction filter Water Bath

Procedure

Enhanced Isolation of Curcumin

To speed up the process of extracting curcumin, the reflux set up should proceed in the microwave. To do this an apparatus must be made that will able to withstand conditions in the microwave. This apparatus consist of two flasks: A polypropylene flask, which was cut to have two open sides, and a glass flask with one open side. A hole was made in a rubber stopper, which will allow a test tube to fit in. The stopper will then connect both the flasks together. The volume of the polypropylene flask was 235cm^3, volume of the test tube was 23.9cm^3, and the volume of the glass flask was 280 cm^3

To set up a reflux the solution will go into the bottom, glass flask, and ice will go into the top flask. So when the apparatus is inserted to the microwave the solution will evaporate and condense in the test tube, due to the ice surrounding it.

For the procedure 50mL of dichloromethane and 20g of turmeric were inserted in the bottom flask. The turmeric was dissolved with the dichloromethane. Ice was then inserted in the top flask to set up the reflux. To keep the dichloromethane from boiling the apparatus was put in the microwave for 10 seconds and removed for 5 seconds. When the ice had melted the top flask is rinsed out and fresh ice is inserted in the flask. This keeps repeating for 30 minutes.

After 30 minutes the solution is suction filtered, and the solution is put into a hot water bath at 50º C until there is a yellow-oily residue left over. This means that all the dichloromethane has been evaporated. Then 20mL of hexanes gets mixed with the solution and the solid that forms must be triturated (grinded).

Then TLC is performed on the sample. 9.7% of dichloromethane and .3% of methanol is mixed in a container. A well is inserted in the silicon slide with a dilute solution of dichloromethane and curcumin. The slide is inserted in the container until the liquid runs all the way up the slide.

To figure out the percentage of curcumin a process called “The area of an irregular object,” is used. The TLC plate is photocopied and bands on the plate are cut and all of the bands are measured at first. Then the major, large band is measured. The

percentage is formed by taking the mass of the major band and dividing it by the mass of all the bands.

Problems that occurred with the design were: First, it did not fit in the microwave, so the top flask was cut down a bit; second, because too much pressure built up in the test tube from the dichloromethane vapors the apparatus blew up in the first trial, so a small hole was inserted on the top of the flask; and finally, because the dichloromethane boils at around 15 seconds in the microwave, the apparatus was only put in the microwave for ten seconds.

Results

Using the traditional isolation method 5g of curcumin resulted, however, by using the enhanced method 7g of curcumin resulted. The mass of all the bands in the TLC was .0487g, and the mass of the major band was .0241g. So curcumin is exactly 49.4% of turmeric. The whole process took 30min. as opposed to 1 hour using the traditional method.

Conclusion

This process is a more efficient way for extracting curcumin and it is extracted in a faster time. Creating an apparatus like the one used I this project does not take any time and it is not that expensive. If turmeric is used to treat patients with Cystic Fibrosis then they can their hands on the curcumin in no time.

At this point in time there is no perfect cure for Cystic Fibrosis. It is a dangerous, deadly disease among people in America and in Europe. There are treatments like gene therapy, anti-inflammatory diseases, healthy diets, and others. These help victims of Cystic Fibrosis, but the victims have to use these treatments numerous times to feel better. Curcumin is potential treatment for the disease and users do have to consume curcumin everyday. There is a proven method to extract curcumin which goes through

numerous steps. This method just described shortens the time the process takes to be fully completed. It yields more curcumin than the traditional method. This process is more efficient and will provide curcumin to people with Cystic Fibrosis quickly if curcumin is approved as a treatment. In the future instead of using ice to set up the reflux, two holes should be made in the microwave creating chilled running water. This will make the reflux process more continuous.

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