Extraction and chromatic separation of plant pigments from tomato paste

In column chromatography a mixture is dissolved in a solvent and poured over a column of solid adsorbent then eluted with the same or a different solvent. This method is often used for preparative purposes; when a relatively large amount of the mixture and the components need to be isolated.

The two main pigments in tomato paste mixture are the yellow-orange β-carotene and the red lycopene. The colors of these pigments are because of the double bonds in their structure. The Lycopene and B-carotene will be separated from tomato paste by using column chromatography. First, the organic layer was separated from the paste by rinsing and drying by addition of sodium sulfate. The remaining solvent was then concentrated by heating before being added to the column in the chromatography. Petroleum ether was used to move the (-carotene down the column where it was desired. The Lycopene was extracted by using a ratio of 90: 10 petroleum ether/acetone.

Chromatography is the science which applies laboratory techniques for the separation of mixtures or molecules based on differences in their structure and/or composition. Chromatography may either be preparative (separate the components for advanced uses) or analytical (for relative proportions). It involves a sample (or sample extract) that is dissolved in a mobile phase (which may be in gaseous form, liquid form or is a supercritical fluid). The mobile phase is then forced through an immobile, immiscible stationary phase. The phases are chosen as such that the components of the sample have differing solubilities in each phase.

A component which is quite soluble in the stationary phase will take more time to travel through it than a component which is not very soluble in the stationary phase but still very soluble in the mobile phase. Due to these differences in mobilities, the sample components will become separated from each other as they travel through the stationary phase giving us the bands we see from the experiment.

Chromatographic separations can be accomplished by using a variety of apparatus, including compacted silica on glass plates which is also known as thin layer chromatography, volatile gases or also known as gas chromatography, paper for paper chromatography and liquids which incorporate hydrophilic, insoluble molecules for liquid chromatography. Column chromatography is one of the many isolation and purification techniques used widely by chemists to obtain pure samples of chemicals from natural sources or from reactions.

If the compounds to be separated are colored then the separation can be monitored visually; although it is more common that the compounds to be isolated from a column chromatography are colorless (which is not the case for our sample). In that case, several means for monitoring the separation progress have been devised. One of the simplest of these involves the collection of relatively small fractions of the eluent in labeled tubes and the analysis of the component(s) of these fractions thin layer chromatography.

Lycopene is the red pigment found in ripe tomatoes and is a natural antioxidant. Meaning, it helps to fight certain cancers and is a C40-carotenoid made up of eight Isoprene units; making it a tetraterpene. B-

Carotene is the yellow pigment of the carrot is an isomer of Lycopene in which the double bonds at C1-C2 and C’1-C’2 are replaced by bonds extending from C1 to C6 and from C’1 to C’6 to form rings, and is also a constituent of the tomato. Each of these compounds is classified as a Carotenoid.

Carotenoids are the organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria. There are over 600 known carotenoids; they are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen). Carotenoids in general absorb blue light. They serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage. In humans, fourcarotenoids (-carotene, -carotene, -carotene, and -cryptoxanthin) have vitamin A activity (meaning they can be converted to retinal), and these and other carotenoids can also act as antioxidants.

In this experiment one will isolate the lycopene from the tomato paste. To do this one will first extract the carotenoid pigments from the paste and then use column chromatography to isolate the lycopene from the other pigments.

Many of our bodily reactions involved in metabolism involve some form of organic chemistry. For example, the breakdown of complex sugars into monosaccharaides and disaccharides is a hydrolysis reaction. As with other reactions, these products are often vital for many of life’s functions. The

simple sugars absorbed are used throughout the body for energy and amino acids consumed in the form of protein help build essential body proteins. One major product of organic chemistry in the body is β-carotene. A precursor to Vitamin A, β-carotene is commonly found in many vegetables and fruits as a pigment and contributes to the yellow-orange color. In the human body, it is associated with retinal (Vitamin A form) formation and helps to maintain proper vision. β-carotene is also converted to Vitamin-A in the intestinal wall and stored in the liver.

This makes β-carotene an important natural product of organic chemistry. β-carotene is also non-polar due and this property would prove pivotal in this experiment. Similar to β-carotene, lycopene is another product of natural organic chemistry. One of the most unsaturated compounds found in nature, lycopene is an antioxidant believed to reduce digestive and cervical cancer risk. Lycopene is not an essential nutrient, such as β-carotene, but is found in many different vegetables and fruits as a red-orange color. It is also used as a food dye in many processed foods.

Although lycopene itself has no use in the body, it is an intermediate in the formation of β-carotene which is a precursor to Vitamin A and can provide nutritional value through this pathway. As with β-carotene, lycopene is also non-polar and this property will be utilized in order to separate the two compounds. This experiment involves the isolation and separation of lycopene and β-carotene from a tomato paste mixture. After the two pigments are isolated, column chromatography will be used to separate them.

Materials:

Tomato Paste Aluminum Foil Silca (chromatographic grade) Glass wool or cotton Petroleum Ether 95% Ethanol Chromatographic column (or a pasteur pipette)

Procedure

First, set-up the columns for the experiment using an iron stand and clamp. Then, prepare the column set up for the chromatography. Press a small plug of glass wool or cotton into a Pasteur pipette with the aid of microscale glass rod to push it to the bottom, and secure this to the iron clamp this vertically. (Note: Check the alignment from front and side.) Fill the column with silica in a depth of 3-4 inches. Tap the side of the column gently with the stir rod to ensure that the silica has settled evenly. Add enough petroleum ether to make a thin slurry.

At the same time, more solvent should be allowed to drip slowly from the column. It is important that there is always enough solvent in the column to cover the silica packing; a column should never be allowed to run dry. Allow the solvent to run through until it is at least 0. 5mL above the silica in the column. Weigh 10g of tomato paste in a 50-mL beaker. Add 20 mL of petroleum ether and stir the mixture with a spatula. Put a small amount of cotton in a funnel blocking its exit. Using the funnel, pour the tomato paste-ethanol mixture in an erlenmeyer flask.

After the filtration proces, the filtrate will be discarded and the residue in the cotton will be used to extract the pigments. In a 50-mL beaker, place the

residue from the glass wool then add 10 mL of petroleum ether. Keep stirring the mixture for about 2 mins to extract the pigments. Filter the extract as before through a new funnel with a glass wool blocking the exit into a clean 50-mL beaker. Evaporate the solvent to about 1-mL volume using a hot plate (or water bath). The solvent must not evaporate completely, if it does, repeat the step where you filtered the pigments before. Cover the beaker with aluminum foil after the evaporation. The oily residue should dissolve in 5. 00mL of petroleum ether and then covered again with aluminum foil.

Transfer 0. 5-1mL of the extract onto the top of the column. Allow the sample to enter the column but make sure it will never run dry. Add 19 or more mL of petroleum ether and wash the sample into the column and collecting the eluted solvent in a beaker. As the solvent elutes the sample you must observed the separation of the two bonds, the color of the pigment bonds and how far are they from each other. Well developed bands of color separated by clear white bands of the absorbent should appear if the column has been properly packed and all traces of polar solvents are absent.

The result of the column chromatography yielded two components that were separated. The first component extracted was yellowish in color, with that being said we could say that this is the B-carotene pigment. The chemical was thought to be B-carotene because its absorption was almost identical to that given in the literature. The second component was deduced to be lycopene. The component was extracted from the column as a red-orange liquid. Both the lycopene and B-carotene were very close to that which was expected.

Column Chromatography is an effective way to separate the Lycopene and B-carotene in the sample. Petroleum ether was used to extract the first chemical and move it down the column. It was a yellowish liquid and the second extract was separated and moved through the column by using petroleum ether. It was an orange liquid . The first extract was B-carotene while the second was the Lycopene, which can be proven with the literature. The factors that will affect the column efficiency are the compactness of the silicate, dimensions of the column, particle size of the adsorbent, the nature of the solvent, and the temperature of the column. With column chromatography, we had an advantage.

Any quantity and any type of the mixture can be separated, and a wider choice of mobile phase and automation is possible. But there are also some disadvantages. It is a time consuming method, more amount of solvents are required, which are expensive and automation technique makes complication not to mention the scenario where the solvent may all be used up and the column dries out in the process requiring one to repeat the whole procedure from the start.