PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 2

Lecture 7

Week 2: Lecture 7: Evaluation, Extraction & Refining of Lipids

Hello everyone, and welcome to the NPTEL course in pharmacognosy and phytochemistry. We are learning about an interesting set of oleaginous compounds called lipids and volatile oils, where we are dealing with fixed oils, fats, waxes, and volatile oils. In the previous session, we studied what volatile oils, fixed oils, fats, and waxes are. In this session, we'll learn a little about their evaluation parameters, as well as some methods for processing fixed oils. So, let's start with the analytical parameters, especially for oils.

And fats. Now, if you see oils and fats, oils and fats are triglycerides in their composition. They contain both saponifiable and unsaponifiable matter. So, if we have to evaluate any oil or fat, a few parameters or physical constants that we can look for are, first, viscosity. Now, in the previous session, we discussed that, depending upon their chemical nature, we can have triglycerides containing saturated fatty acids or unsaturated fatty acids. Those containing more saturated fats tend to be solids. And those containing a good amount of monounsaturated or polyunsaturated fatty acids tend to be more fluid.

Also, depending upon their nature, we could classify them as drying oils, non-drying oils, or, in some cases, semi-drying oils, depending upon their ability to oxidize and form films. So, there are some oils which are very viscous. And there are some oils which are very fluid. Some oils, such as coconut oil, solidify. You can evaluate them based on their viscosity. The second parameter is specific gravity.

So if you see most of the oils or oleaginous ingredients, they have their tendency to float. That means they have a specific gravity which is lighter than that of water. Now, comparing between your oils and fats, your oils tend to be lighter than water, but they'll be definitely heavier when you compare them with your fats and waxes. Fats and waxes, the specific gravity tends to be a little less. So if I'm talking about a fixed oil, I might come across a specific gravity value ranges somewhere close to

0.99 0.98 0.96 whereas when I am talking about your waxes or fats I might come across something very lighter like 0.95 in some cases the value may be till 0.88 and so on. So the specific gravity changes depending upon their nature. Now, when it comes to refractive index, definitely they are more refractive when compared to water with on an average, the refractive index values ranging somewhere between 1.42 to 1.47. Now, here also, you will see that compared to your fixed oils, your volatile oils are more refractive or have a higher refractive index. So if I'm talking about 1.46, 1.47,

The higher range is generally for the volatile oils. And the last evaluation parameter what you can check is solidification and congeal point. Now, depending upon how much saturated fats are there, how much unsaturated fats are there. And it's a blend. It's not going to be a single compound.

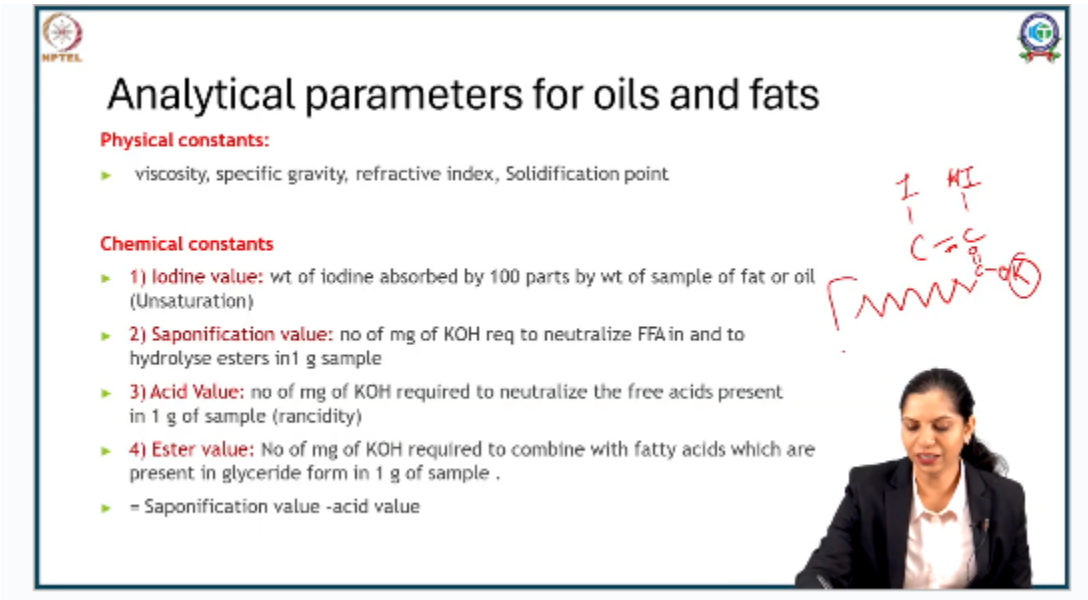
And as a result, every oil will have its own solidification and congealing point. And that's the evaluation parameters. Now if you go to see chemical constants. They help us determine numerous criteria. Let's start with something as simple as iodine value.

Now, we know that iodine value is actually the amount of iodine absorbed by 100 parts, say 100 grams by weight of sample, which could be a fat or oil. So why would any sample absorb iodine is when particularly there is an unsaturation. So across this unsaturation, your iodine might get added and more the unsaturation, I'm sorry, more the unsaturation, higher will be the iodine value. So the iodine value measures the degree of unsaturation. Higher the iodine value means higher unsaturation.

So if I am again trying to balance between oils and fats, who will have the higher value? Any guesses? So it will generally be oils which contains more amount of PUFA that will

have your higher iodine value. Now coming down to saponification value. Saponification is a process where you create soap.

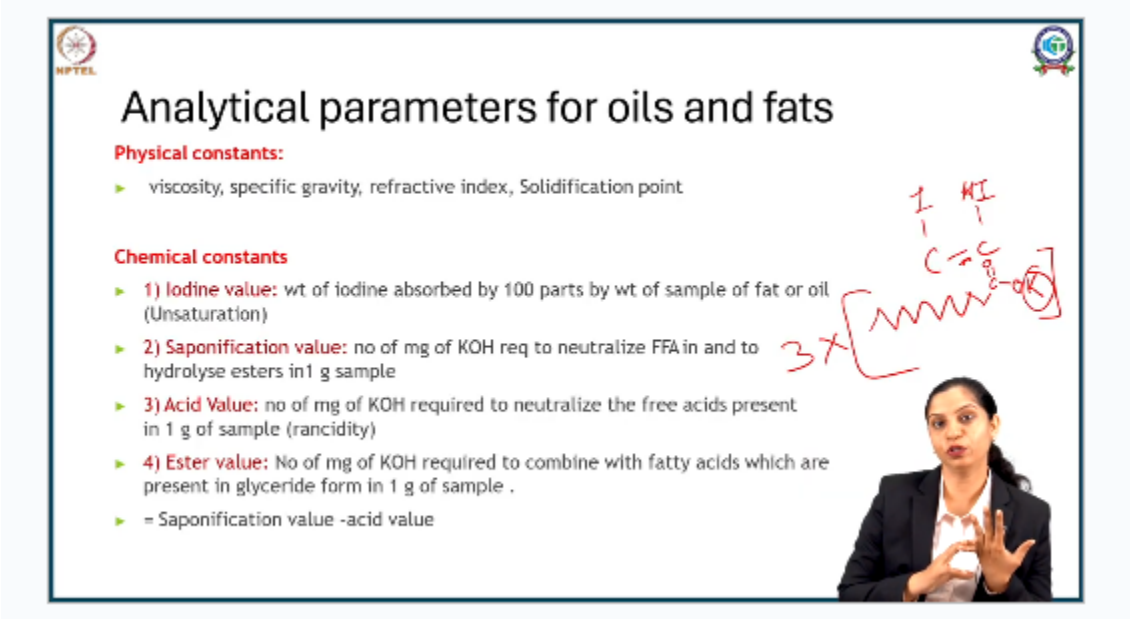
from a free fatty acid. So we know that we have triglycerides in our fixed oils and triglycerides in our fat. Now our intention is to take them and convert those free fatty acids into soaps. Free fatty acid soaps are as good as representing say for example I am having a fatty acid. And I put it as say for example COOH.



So rather than OH I will have a O potassium or sodium depending upon what salt I am adding here. So if it's a triglyceride this 3 happens into thrice. So three times it will absorb or three times it will take. So how much milligram of potassium hydroxide actually is required to neutralize the free fatty acid, which comes from hydrolysis of this triglyceride is called as the saponification value. This is generally done as elevated temperature or little heat might be applied some in some cases where you want to break the triglycerides.

On the other side is if you just want to determine how much free acids it contains, the next value is called as acid value. So apart from triglycerides, in some cases, the oils will also contain free fatty acids. Now, free fatty acids or determination of free fatty acids is very important because more the amount of free fatty acid, the higher is the chance that your oil will turn rancid. So rancidity parameters can be directly correlated with free fatty acids. And imagine if this free fatty acids are the unsaturated free fatty acids.

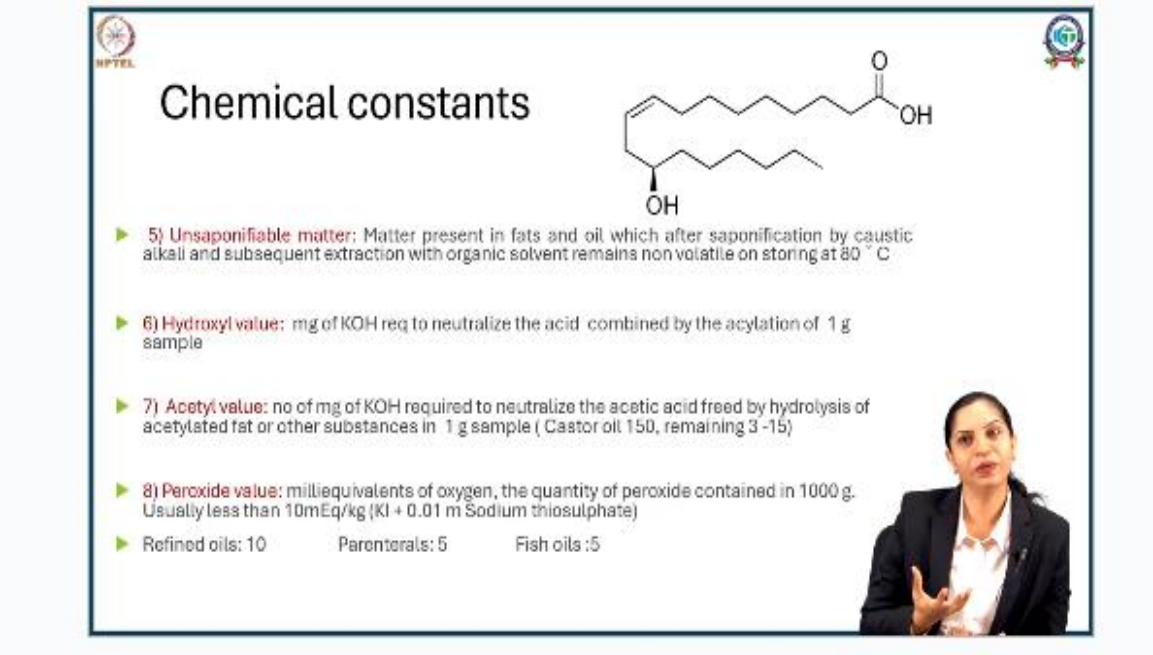
In that case, you will have even more. More chances of rancidity occurring. So, in order to get an idea of whether the oil is prone to rancidity or not after saponification, one can even determine a simple test—just don't hydrolyze the triglyceride, just react. Potassium hydroxide with your oil as such at room temperature—whatever amount of potassium hydroxide or whatever milligram of potassium hydroxide is required in the titration to neutralize the free fatty acids present in the sample, especially one gram of sample—that is what is called your acid value. Now, the next one is the ester value.



So, if you see the saponification value, it gave you the amount of fatty acids present in your triglycerides plus free fatty acids. So, saponification is actually an indicator of triglycerides, which had three times your fatty acids, if I may say, plus it also gave you an indicator of free fatty acids. Now, if you determine the acid value, your acid value is just going to tell you about your free fatty acids. Now, if you subtract the free fatty acids or if you subtract the acid value from the saponification value, you will come to know how much acid is involved in esterification. So, what I'm doing is I'm just taking the saponification value.

OK, and from the saponification value, I'm going to deduct the acid value. This is the acid value. What I'm going to get is this. How much fatty acid is actually involved in ester formation? And that is what is called the ester value.

So in terms of chemical constants, you have iodine value to give you saturation. Saponification value tells you how much fatty acids are involved, both in free form as well as triglycerides. Your acid value will just tell you the amount of free fatty acids, and that can correlate to your rancidity. Your ester value is simply how much fatty acids are involved in triglyceride formation, which is nothing but the difference between saponification value and the acid value.



The next set of parameters are what is called unsaponifiable matter.Now, whenever you take your oil, you might have heard that this oil contains vitamins, this oil contains oryzanol, this oil contains something which is very healthy, such as pigments, antioxidants, or some of you might even have heard of tocopherol, vitamin E derivatives. So this oil is rich in vitamins. So what are these? The compounds apart from fatty acids which do not undergo saponification are all termed together as unsaponifiable matter. So unsaponifiable matter is the amount of matter or the weight of substance which does not undergo saponification.

And then it is lipid soluble. So after the soap is formed, what you need to do is take your organic solvent. Put it in the saponification reaction, and whatever has dissolved now is all

your lipophilic ingredients which have not undergone saponification. So all this you will take and maybe heat it. You will get what is called unsaponifiable matter.

So good example of that is your lignans which is present in your sesame, your vitamins, tocopherol derivatives, vitamin A, vitamin E, your pigments. And your sterols, all of them are categorized as unsaponifiable matter. The next set of chemical constants which you determine for oil are your hydroxyl value, acetyl value and peroxide value. So what is this? So say, for example, your compound such as this is an example of ricinoleic acid.

And now ricinoleic acid has a hydroxyl group. I want to determine how much hydroxyl groups are there in my sample. So what I do is I maybe react it with acid. Say, for example, I reacted with acetic acid and then as a result, I convert this into an ester. OK, so I convert this into an ester.

So what happens in the process is I have acetylated it. Now, later on, I will hydrolyze and I will see how much acid reacted. The amount of acid reacted can tell me how much amount of hydroxies are present. And that is what we call it as hydroxyl value. So hydroxyl value is the amount of KOH that is required to neutralize this acid.

which was combined by acetylation to 1 gram of sample. Now, in some cases, you can also determine what is called the acetyl value. The acetyl value is generally for fat samples. So it is the number of milligrams of potassium hydroxide used to neutralize the acetic acid freed by hydrolysis of acetylated fat or even one gram of sample.

For example, in castor oil, the value might be as high as 150 because it contains ricinoleic acid. But in some oils that do not have hydroxyl groups commonly present, the acetyl value may be as low as 3 to 15. The last value is also important and is called the peroxide value. Now, what happens here is imagine your unsaturated fatty acid. Over time, it will tend to form epoxides.

These epoxides might even form peroxides. In this process, you will get peroxide derivatives. Eventually, the molecule will break, giving you aldehydes and ketones. We term that as rancidity. So peroxide is an initial step toward rancidity, where oxygen is taken by the oil to convert it into smaller fragments.

So how do you determine this? The peroxide value is done or determined by iodometric titration. So here, what is done is maybe— You release the peroxides, allow them to react with acid in the presence of potassium iodide, and the amount of iodine released can be titrated with sodium thiosulfate. Now, since these values are very low, rancidity is very low.

If you see comparatively, in most cases, what we have done is take one gram of sample. But for rancidity or peroxide value determination, the amount of sample required is pretty high. So if you see refined oils, it's good. But if you want to use it for parent drills, the oil should not be rancid at all. So for, you know, like 1 kg or 1000 grams of sample, the rancidity—the peroxide value—should be as low as 5.

Even for fish oils, it should be low for safe consumption. The next set of values is also a rancidity value, where you can determine how many aldehydes are formed. Like we saw previously, whenever the peroxide breaks, it will eventually give you an aldehyde. Now, this aldehyde can be a small aldehyde like— you know, ephedrine or malonaldehyde being formed.

CRIS test is a test which allows a reaction between malonaldehyde or epihedrine aldehyde, phloroglucinol. When reacted with oxidized fats, it will give you a reddish coloration and that reddish coloration will tell that whether, you know, it's almost on the verge of rancidity or is it oxidized or not. The other sets of values gives you interesting what are called fatty acid determination. The first one is a reichert meissle value. Now, reichert meissle value is a value which will tell you how much volatile

Water soluble fatty acids are there in your sample. So what you have to do is you take a sample, you steam distill it. Now when you distill it, all the volatile components will distill out. You collect the distillate and that distillate is titrated with potassium hydroxide. Now, once the distillate is titrated with potassium hydroxide, what you get is a set of water soluble volatile acids which have come out during the distillation process.

So that value, if you take 5 gram of your sample, the amount of KOH which is required to neutralize the fatty acids is determined or is known as your reichert meissle value. on the other hand polenski value is something which is used to determine what are called as what

are insoluble but steam distillation so in some cases what happens is when you distill your 5 gram of fat you might come across a distillate which separates into a layer And if that layer is a water insoluble layer and when you want to titrate it, you use alcohol to dilute it so that it dissolves. So when it dissolves and when you titrate it with a N/10 KOH, what you get is a amount

of water-insoluble but steam-distillable fatty acids. In that case, you get what is called Polenski. So, this sample is hydrolyzed and then subjected to distillation. So, you get the amount of fatty acids which have been involved in the triglyceride formation as well. The last one, if I have to determine how much rancidity is actually present, I can do it colorimetrically using what is called the anisidine value.

So, I take my oil sample, solubilize it, and then add anisidine. Now, this anisidine reacts to form a colored complex, especially with ketones. This is generally used for the determination of fish oils such as cod liver oil. And if I see the absorbance at about 350 nanometers, the intensity of absorbance will tell me how much anisidine is actually present.

Now, let's go to the next step. After we finish the evaluation, how do you extract oils? Now, for the purpose of extraction of fixed oils, it can be done by three simple techniques. The three simple techniques include cold press. You must have heard of mechanical press or you must have seen a sugarcane juicer.

So you have machines which are a set of grooves, and you put your raw material into it. If the raw material contains sufficiently high oils—say, for example, your castor, your groundnut, your sesame—they contain almost more than 30% of their weight in oil. So when the seeds are so high in oil, if you just put them in your ghani or your press, you will see that the oil extrudes. This is easily done, and when the oil content is high, you can use a cold press. But if the oil content happens to be a little low, you have to facilitate the process of extraction, and that facilitation can be done by heating.

So what you can do is either steam the seeds. What happens during the steaming is the cells swell, the oil globules congeal, and eventually facilitate. So, in the same way, or in some cases, it is done at elevated temperature, or even your mechanical presses—the plates are

at higher temperature. So, at higher temperature, it's called a hot press, and in the hot press, the meal also— the seeds or the plant part from which the oil is to be expressed gets heated.

Because it is heated, all the oil kind of congeals, collects together, easily separates out, and you can have a higher yield of oil coming out. The last process is solvent extraction. This is a done for substances which contain a very small quantity of oil. So even if you squeeze it, press it, or even if you heat it, if your plant is not releasing the lipid substance—one good property of solvents we know is 'like dissolves like.'

So what you can do in this case is use lipophilic solvents such as hexane or petroleum ether, which are very inert. And using these solvents, if you dip your drug into them, all the lipids will dissolve in these solvents. Now you filter it out, and from that filtrate, you will evaporate the solvent. Now, hexane, petroleum ether, and all these solvents have a very low boiling point. They are known to be volatile, whereas your fixed oils have a very high boiling point, sometimes even exceeding 250 or 300.

So they can easily withstand temperature, and when you heat them, your solvent will evaporate, leaving behind your oil. So these are a few easy ways the industry uses to extract fixed oil from samples. Now, once the fixed oil is obtained, in some cases, it is still considered unfit for consumption and requires subsequent treatment, which is called refining. So maybe you have heard of this term: refined oils.

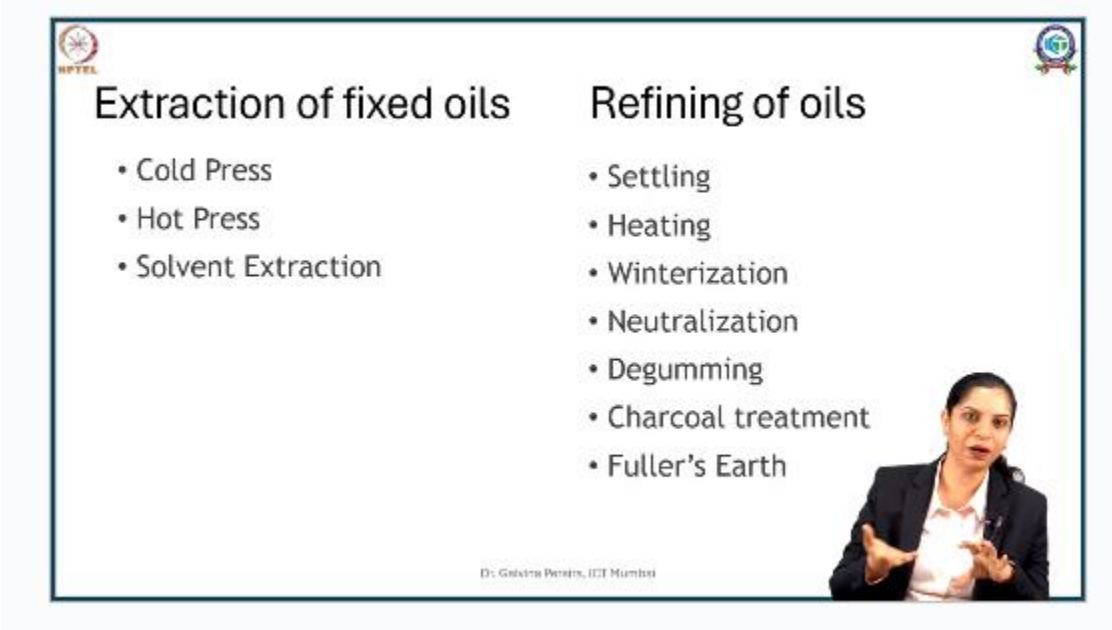
So let's see what refining is. Refining of oils can start with a very simple step, such as settling. For example, if I take coconut, specifically the coconut endosperm, which is dried copra, and press it, sometimes what I get is something like coconut milk. So what happens is it will take time for the oil and water to separate.

Now, in some cases, there are some solids which will make my oil turbid. I need a clear oil. So what I will do is allow those particles to settle. So separation and settling we call it aging of oil. So after expression, your oil is kept in a settling tank for a while to let the water phase, the moisture, or the solid particles separate out.

This process is called settling and is often the first step after the cold oil or the hot-drawn oil is obtained. Once that is done, in some cases, heating is also performed. Now, heating

serves the purpose in cases when there is moisture; heating will remove the moisture. In some cases, when there are odorous principles present and this odor is not desired, heating will help us remove those odorous principles.

Heating can help us in denaturing enzymes. So if you take, for example, rice bran oil or castor oil, these oils contain lipolytic enzymes such as lipases, and lipases have the ability to hydrolyze and degrade your triglycerides. Now, if this happens, I am at a high risk that my oil will turn rancid. So heating is done to denature the enzymes and to dry out any traces of moisture present.



So the oil is taken, heated at to about 200 to 50 degrees Celsius where it is generally stable. So the water components vanish, the enzymes get denatured and any other unstable impurities is there. In some cases, it might also kind of coagulate with that protein and settle down. The third process which is used in the refining is called as winterization. In some cases what happens is other lipophilic ingredients which are not required in the oil also enter in.

So in that case what I need to do is I'll take my oil and slowly slowly cool it down. Now what happens when I cool it down is there are some impurities which will become insoluble. Why? Because their melting point is in that range. So, say for example,

If I cool something to zero degrees Celsius, my castor oil normally doesn't freeze at zero degrees Celsius. But if my castor oil contains some ingredients which will solidify, whose melting point is close to zero degrees Celsius. So when I am dropping the temperature to subzero degrees Celsius, I'll see some ingredients with those kind of melting points solidifying once again. So during the solidification process, I get rid of estearin and other solid impurities which would otherwise precipitate in my oil. So that is called as winterization.

This is generally done for fish oils also. Then, neutralization. Neutralization refers to the treatment of free fatty acids. Now, we know that fatty acids are involved in triglyceride formation. But in some cases, they remain available as such, rendering the oil slightly acidic, and these free fatty acids make it prone to rancidity.

In order to get rid of these free fatty acids, what you can do is treat it with a lye, such as sodium hydroxide or potassium hydroxide, or just treat it with sodium bicarbonate if it is very mild. This treatment helps in removing or neutralizing the acids that are present. So, neutralization is a treatment often given to remove or get rid of free fatty acids. Next is degumming. Often, you will find that components such as lecithins and phospholipids enter in and act as emulsifying agents.

So, they form a gummy exudate or are left as a gummy layer. Now, what we can do is, in some cases, treat it with water because carbohydrates are water-soluble. In some cases, it can even be treated with citric acid or other acids so that those phospholipids are broken down. In some cases, this can even be done with enzymatic treatments. So, all of this will get rid of your phospholipids, gums, or mucilaginous substances, which would otherwise interfere with the clarity of the oil.

Once that is done, the last part is you can do a charcoal treatment wherein most of your pigment impurities are absorbed by the charcoal columns. So you can pass your oil through charcoal columns and do that, or even use fuller's earth or silica. These kinds of beds are

created, either charcoal beds or silica beds, and oil is passed through them. So all the coloring impurities, minute traces, they are all adsorbed, and once they are adsorbed, your oil is pristinely clear and clarified. So it's passed through that, then through good filters, and what you get is your pharmaceutical-grade refined oil.

So what you learned today are the chemical methods of evaluation of fixed oils, extraction, and the refining of fixed oils. So here are a few more readings if you wish to proceed with studies on this. And thank you, everyone, for your patient listening.