PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 3

Lecture 12

Week 3: Lecture 12: Carbohydrates based drugs

Thank you. Hello everyone, and welcome to the NPTEL course on pharmacognosy and phytochemistry. This week, we are delving into the world of carbohydrates. In this session, we will discuss two industrially important carbohydrate derivatives: starch and pectin. Starch is a compound called an ergastic cell content, meaning it is produced within the plant cell as a result of photosynthesis when carbon dioxide and water are converted into glucose.

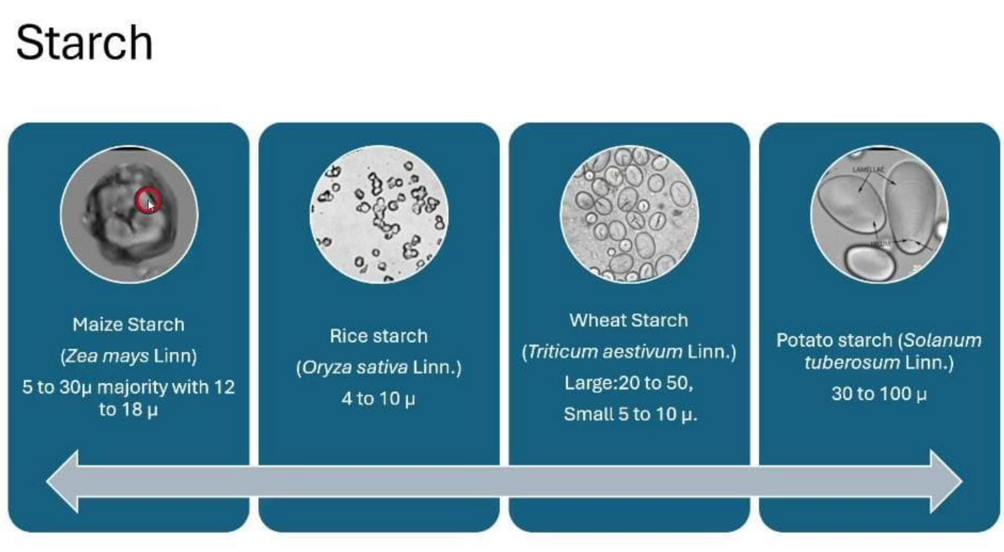
This glucose accumulates and increases osmotic pressure, and as a result, the plant converts this glucose into storage granules. Within the organelles called amyloplasts, they are stored as starch. Starches are nothing but polymerized forms of glucose stored in plants. Naturally, every plant stores starch, but for commercial extraction or utilization, starches are generally obtained from grains. They are also obtained from tubers, which are dug up and contain enormous amounts of starch. These can be grains such as maize, rice, wheat, or underground tubers such as potato and tapioca, also called cassava. The second example is pectin.

Pectin is a carbohydrate present in fruits. These fruits may include orange peel, apple, sugar beet, guava, mangoes, or papaya. When you consume these fruits, you get a silky smooth texture. This slimy texture is attributed to pectin-like compounds. So, let's first discuss starch in detail.

So starch, as I said, is commercially obtained from grains as well as from tubers. Let's see a few examples of starches which are there in commerce. The most abundant one is maize starch, which is obtained from corn or, botanically, we call it as *zea mays*. The maize starch appears like a polyhedral shape with a central hilum and some cracks or fissures emanating from the hilum.

Now what happens here is when your plant is converting the glucose into starch granules, you will see a point of attachment to the amyloplast, and that's where your hilum is. Now gradually, over a period of time, the sugar molecules build in and they grow in size. Not only do they grow in size, they grow exponentially. as the plant ages. So the plant goes on accumulating them in the amyloplast, and the way in which they are accumulated is responsible for a typical appearance.

Now this appearance also helps us in diagnosis. So maize starch is characterized by a polyhedral outer border, an inner hilum, and some fissures emanating from it. Generally, with a particle size or what you can say as an average diameter around 5 to 30 microns, with most of the particles falling between 12 to 18 microns. Now, as compared to maize starch, the second starch is rice starch, it's very tiny in appearance. So in terms of size, they are just 4 to 10 microns, but these are also polyhedral, meaning they have sharp angles.



Now these are polyhedral but tiny. As compared to maize starch there is one more difference between maize and rice starch is rice starch are generally found as compound granules that means they will be found as agglomerates. And in some cases, when you zoom it, you might find some hilum. But in most of the cases, they are too tiny to observe the hilum. The third case or the third abundant starch is your wheat starch.

Wheat starch comes from wheat. That is *Triticum aestivum,* which is also a Graminaceae family member like your rice. And the particles here are not polyhedral, but they are slightly oval. They are oval shape and slightly flattened.

They are large as compared to the previous two that is 20 to 50 micron and the smallest ones very tiny ones interspersed in between they are about 5 to 10 micron in size. One of the biggest starch particles you can see in commerce are potato starch. They grow very big from 30 to 100 micron in size and they are slightly elliptical with a hilum or a dot, which is actually the point of origin or attachment of your stars. And later on, you can see some striations.

Now, this is because what happens is, as the potato tubers grow, the moisture content and the ratio of amylose to amylopectin changes. And that gives a slight difference in the refraction between these two. And that is the result you see as numerous striations or what we call lamellae in this case. So, starches are generally made up of polymers of glucose, but depending upon their linkage, you can classify these polymers as amylose and amylopectin. Amylose is actually a linear polymer, whereas amylopectin is a branched polymer.

So, if I have to give the difference between these two polymers making up your starch grain, amylose is the tiny component that is linear but just comprises 20 percent of most of the starches, and in some cases, it might be even less than 5 percent, so it depends again on the source of the plants, but this is generally the minor component. Now, amylopectin is the major component, the branched component, adding up to thousands of sugar molecules, and because it is branched, you can see amylose has what is called 1,4 linkages or alpha 1,4 linkages between the glucose. Here, apart from 1,4 linkages, you also see 1,6 linkages between the glucose. So, amylopectin has two types of linkages: alpha 1,4 and alpha 1,6, whereas amylose just has alpha 1,4 linkages.

Now Saying that, amylose is actually stored in the plant in the form of a coil. So, this polymer, this linear polymer, is actually coiled, and when you boil your starch, it opens up and hydrates. And amylopectin, you will see, because it is branched, it is a little resistant to swelling, and that is also a reason why it is not readily dissolvable or soluble. So, you see, in terms of solubility, amylose quickly uncoils and dissolves.

Hydrates and becomes solubilized in water, whereas amylopectin is considered to be water insoluble. At room temperature, they have poor solubility, but as you increase the temperature, their solubility in water increases. Now, because of the branching, amylopectin is hydrolyzed, which is very resistant to hydrolysis. So if you try to break amylose versus amylopectin, you will see breaking amylose is much easier because it's linear. But if you try to break amylopectin, because it is branched, the enzyme doesn't fit in properly due to the branching.

As a result, it kind of stereochemically hinders the hydrolysis, preventing its digestion by acids as well as enzymes. Now, one more test you can do to check amylose and amylopectin is their reaction with iodine. Whereas both of them form charge-transfer complexes with iodine, the nature of the reaction is slightly different. The nature of association is slightly different. So amylose forms a complex which gives you a bluish-black coloration with a good absorbance between 600 to 620.

Whereas if you see amylopectin, it is more of a reddish-brown colored complex with a UV maxima or absorbance of about 540 nanometers. Now, how do you extract starch? Let's take an example of maize starch, which is widely used commercially. So what you need to do is take those grains, especially maize grains. And now you know that those maize grains are hard and stiff.

So what is done is they are washed in water. Sometimes they are kept in warm water for 2 to 3 days to allow them to imbibe water so that they swell. Now one problem with that is if you allow something which is rich in starch to be in water for two to three days, it is prone to microbial fermentation, and that is something we do not want. As a result, the industry uses two approaches. One thing they do is bubble sulfur dioxide gas through the broth, which is the water containing the corn. And what is done is the slightly acidic conditions are maintained, and as a result, no microbial fermentation occurs. In some cases, within that water itself, about 0.2% of sulfurous acid is added to give similar results.

So this will prevent the corn, especially the starch which is present in the corn, from fermenting. Now once the kernels are nicely swollen, they are crushed or milled gently. So when you crush or mill gently, what happens is the outer hull as well as the germs start separating out, and the starch separates. Now one phenomenon observed here is you will see most of the germs floating. So they are tiny, and they start floating.

These are actually skimmed or collected separately to get what is called germ oil. Now this germ oil is very rich in vitamins and is used in cosmetics as well. Now this water or this slurry, which is now there containing your starch, also dissolves most of the monosaccharides, that is glucose and other sugars which are present. It will dissolve the plant minerals. So that your essential minerals which are present and also the soluble proteins.

So this slurry is very rich in nutrients and it is one of the slurry which is used in preparation of antibiotics such as penicillin. This slurry is referred to as corn steep liquor. Now this coming back to our solution. Now we have a corn steep liquor which is going into your pan. fermentation industry now this starch is there is loaded or stuck in between gluten and you know gluten is a little proteinaceous matter so what is done is this gluten is also allowed to hydrate and it will form sticky mass now this sticky mass is washed and as you wash the gluten remains intact whereas the starch grains which are entangled within the gluten gets washed

So all these washings will have most of your starch and some amount of small particles of gluten. Now comparing their density, starch is much much denser than gluten. So what is done is you allow it to wash or be washed multiple times till all the starch is removed. And then you allow it to settle. Initially the starch will settle and that will be followed by your gluten.

Now industry has also used one trick is they use a trough. which is slightly longer as well as slightly inclined. So what happens is initially on the initial part of the inclined plates, your starch will settle, whereas on the last or the end part of the plates is when your gluten precipitates. So you can take the initial plates and get your good quality starches from there. No matter how much you try this starches will still contain minor traces of other impurities and might require further washings and purification.

So once this is done the starch is dried generally by centrifugation or you can put it in the tray dryers or flash dryers or moving bed dryers which are then subjected to little high temperature but gradual so that the process of gelatinization does not happen to get your starch now other drugs such as potato which doesn't contain much of the gluten the process is much much simpler you have to grind it just separate the cellular debris and pass it through a fine mesh what is going to happen is through that fine mesh your tiny starch particles will separate down just give it a little treatment and you will get your purified starch done Now, once the starches are obtained, industry uses and converts them into much, much more durable and more useful products. And some of these useful products are your modified starches. So let's see a few examples of modified starches.

One is oxidized starch. Now, these are all edible. So this potato starch is oxidized with sodium hypochlorite and a slight polar variant is created, developed and this polar variant is then used in gels and paste to maintain a good consistency. These E numbers are the food additive numbers you generally see on the food packets.

So if you see any of these numbers you are dealing with starch additives. Another is E1412 which is di-starch phosphate. So again this is made polar by adding phosphate moiety to it and it gets a unique property and that it acts as a thickening viscosity and stabilizing agents so that your food particles don't sediment very easily and you get a nice ketchup or thick consistency to it. Another derivative is your E1420 which is your starch acetate.

Now, this is also a stabilizer but a little phobic stabilizer. In this case, it is treated with acetic anhydride to convert it into acetate, and it is also used as a stabilizer as well as a thickening agent. Now, there are some which are called modified starches, and here one which is abundantly used is E1450, which is starch sodium octenyl succinate. Now, this is done or prepared by reacting the starch with octenyl succinic anhydride, and this has good utility as an emulsifier and an encapsulating agent. Now, this also gives you a little bit of hydrophobicity.

Now, let's go to a completely hydrophobic starch. This can be done—imagine if you have those hydroxyl groups of glucose and you esterify them with fatty acids, especially by creating modified fatty acid methyl esters, and then add them to your starches. What happens is you get a comparatively hydrophobic starch, and these starches have been used to create a good water-repellent layer. This has also been used to create, you know, a kind of waterproof paper or dustings when you want it. The last one is sodium polyacrylate starch.

This is when you have polyacrylic acid being used as a cross-linking agent. This is used again in cosmetics as an emulsifying agent, and it has a very unique ability to absorb many-fold its own weight of water. So, this has also been applied in some cases of baby diapers. So, those are many uses of starches. Apart from that, if you see native starch, you have it as a nutritive, you can use it as a demulcent, protective, and also an absorbent. Now, if you want to replace your talcum powder with something more subtle or gentle, this has been used for dusting, but you have to take care of the moisture content. Now, we know that iodine reacts with starch to give you a complex, especially if you remember those amylose coils.

So in that cases when people have suffered from iodine poisoning, the starches have been given so that they can chelate, create a charge transfer complex and encase it in the starch matrix so that the poisoning is not spread. Now, this is excellent binding and disintegrating agent used in tablets and also used as diluent when you don't want the drug to absorb too much of moisture. When you want to create a free flowing powder, generally starch is used as a diluent. Now, nature of starch is different. Depending upon the drug, the starches vary.

starches can be used as a identifying or a diagnostic agent. It is used to prepare liquid glucose. Once you hydrolyze it, you break it into glucose. It can be converted into dextrose and dextrins with the help of microorganisms. And it is also used in industry for sizing paper and cloth.

So these are many many but still unlimited uses put together of starch. Now let's go to the next polymer and that is your pectin. Now when you say starch it was a homopolysaccharide. Pectin is a heteropolysaccharide and it is there as a heteropolysaccharide containing a variable number of sugars. Now the amount of

Of sugars, maybe in thousands. And if you see the molecular weight, it ranges somewhere between twenty thousand to four lakh. So this pectin is naturally a component. If you see the higher plants, you'll find it in plant walls, especially in gymnosperms, pteridophytes, and bryophytes. So primitive plants have it in a scanty ratio.

But if you see most of the higher plants, it will be there, and they're abundantly found in fruits. Commercially, pectin is obtained from orange peels and apple pomace. The reason is, if you see the composition, once the oil, other ingredients, and moisture are taken out, you will see citrus contains a very high percentage—almost 30 percent of its dry weight in terms of pectin. Now, this pectin you can visualize. So when you take an example of a banana or any raw fruit, when the fruits are raw, they are very stiff and very hard. The reason is you have pectin in a highly polymerized, cross-linked form, and that polymerized cross-linked form is called protopectin.

As the fruit matures, some of these bonds break up, and then from a highly cross-linked structure, you have something which is less cross-linked, and as a result, the fruit softens. And that is what we call or refer to as pectin. But if you see a banana post-maturation, when you call it an overripe banana. So what happens in that case is there are enzymes causing this deterioration. So from protopectin, they made pectin.

Now from pectin they will convert it into pectic acid that is a breakdown product. The pectin is no longer able to hold shape and converted into small small units of pectic acid. And as a result your fruit becomes very flaccid and soft. So that is how or that is the role of pectin. So what exactly is pectin?

So if you see pectin, this is made up of linkages. Like I said, it's a heteropolysaccharide. But let's start with something simple. So imagine first a homopolysaccharide of alpha 1,4-galacturonic acid. Galacturonic acid is uronic acid derivative.

So your CH2OH gets converted into COH. Now what happens in the process is if we methylate it you get what is called esters. So these uronic acid derivatives may get esterified. So majority, the biggest part of your pectin, say about 65% of your pectin is actually methyl esterified derivative of galactouronic acid.

We call it homogalacturonan. Now, this is 65%. So, what is the remaining percent? Now, in some cases, imagine this polymer growing slightly longer, increasing in domain, and in between, the sugars are replaced by rhamnose. Now, if it is replaced by rhamnose, what happens is you get an alternate chain of galacturonic acid plus rhamnouronic acid derivatives.

So, alternatively, this is what is called rhamnogalacturonon 1, which is the main part and consists of another 20 to 35 percent. So, the main is pure homogalacturonan. Then you have your rhamnogalacturonan 1. Now, this is not all. Now, just imagine, apart from rhamnose, there is branching.

Now, this branching is done with the help of different sugars. You can imagine about 11 different types of sugars, including xylans, apios, arabinos, and so on. So, many of the sugars go on cross-linking and form other side chains. So now, because they are side chains, they are going to be more branched, more cross-linked, and that is what is called rhamnogalacturonan 2. So, this comprises another 1 to 8%, and a small percent, which is intervened by xylose, is called xylogalacturonan.

So all these four portions put together make up your pectin which is a heteropolysaccharide and is a big bulky polymer. Now this is often found in association with cellulose and hemicellulose along with the proteins and needs to be separated. So let's discuss how we extract pectin from the plant source. So like I said commercially it is obtained from orange peel because it has about 30% of its dry weight. So we take this lemon or citrus peels, we boil it because pectin has a good water solubility at higher temperature.

We boil it at a little higher temperature, close to boiling point, say about 90 degree Celsius for half an hour. Now, during this process, in order to facilitate extraction of pectin, what you can do is you can maintain the pH on a little acidic side. This will promote hydrolysis, like little conversion of small molecular weight pectin and those will be easier to extract post hydrolysis so you can do that with help of organic acids such as lactic, citric or tartaric acid so once that is done still majority of the part remains within the citrus so you can completely squeeze the peels remove out how much ever water content you can so you will get a solution which is little colloidal and turbid centrifuge it and you will get your clear solution.

Now this clear solution does not only dissolve pectin but also has the ability to dissolve small monosaccharides as well as proteins and in some cases your starch particles also come along. So what is done is it is subjected to enzyme hydrolysis. We call it clarification. So this clarification is done by amylose which deteriorates the starch and certain proteases which deteriorate the proteins. Now you have a very clear solution containing your pectin but the enzymes are there.

So, in order to inactivate the enzymes, the solution is heated one more time so that those proteins get denatured or inactivated. So, after inactivating the enzymes, what is done is some pigments also make their way. In order to get rid of the pigments, you give it a good charcoal treatment. So, it is decolorized with the help of activated charcoal and then filtered. Now, this filtrate contains dissolved pectin, and this dissolved pectin, being a carbohydrate, can easily be precipitated by miscible mid-polar organic solvents such as alcohol.

So, you can use methanol, ethanol, or even acetone. In these solvents, these carbohydrates are insoluble. So, the moment you add them to the water, you're going to have a precipitate of pectin coming out. Now, this is slimy and mucilaginous. So, to remove the water which is adhering to it, it's washed with more water-soluble solvents and then dried in a vacuum to get rid of the moisture.

Once that is done, it is pulverized and stored in airtight containers. So, pectin is a yellowish to white powder. It's odorless, Mucilaginous. Now, like we discussed previously, if you remember this methylation.

Now, depending on how much methylation has occurred, you can divide your polymers into high methoxy pectin and low methoxy pectin. So, what is the difference? High methoxy pectin contains more than a 50% degree of esterification. That means in terms of your uronic acid. Most of the residues—more than 50 percent—have been methoxylated, whereas in low methoxy pectin, less than 50 percent have been methoxylated.

What is the difference that the degree of methoxylation or degree of esterification creates? High methoxy pectin is more water-soluble compared to low methoxy pectin. As a result, they vary in their gel formation properties. If you deal with high methoxy pectin, they require—for their gelation—a combination of acidic pH and sugar. Only when these two are present will form gels.

Otherwise, they will not gel properly. Whereas, when dealing with low methoxy pectin, you will require a metal ion such as calcium, and maintaining an acidic pH is not necessary. Here, gelation can happen over a wide range of pH. Now, comparing the gel strength: high methoxy pectin gives you a stronger gel—imagine consuming gummies—whereas low methoxy pectin forms very soft gels. Imagine the glazings on cakes or pastries. They form very soft gels with a nice, smooth, pliable consistency—more elastic—whereas high methoxy pectins give more friable gels. Now, here are the applications of high methoxy pectin in jams and jellies.

Generally, because they require high sugars for their gelation, they give you a very firm gel, whereas in fruit fillings, the soft glazings or toppings mostly come from the low methoxy pectin derivatives. So, both high methoxy and low methoxy have been used in food, industrial, as well as pharmaceutical sectors. In food, they find applications as thickening agents, viscosifiers, and pH stabilizing agents for food especially in dairy products and low-calorie products because of their nice carbohydrate and alternating soft nature they have also been used as very good emulsifiers.

Not only that, they have also been used in the design of drug delivery systems. So, especially your gastrointestinal ones. Now, they are water-soluble fibers. They are water-soluble carbohydrates which create a viscous solution. So, they decrease the absorption of nutrients like carbohydrates.

They decrease the absorption of cholesterol or lipids. And they have also been proven beneficial in cancer treatment because they fall into the category of prebiotics. So, they can also be used as safe for consumption, edible films, coatings, foams, and even paper substitutes. Now, when you see bioengineering, pectin has found its application especially in tissue engineering, wound healing, gene delivery, and also in novel drug delivery systems. Not only that, pectin is derivatized, you can have different degrees of esterification.

Some of them add functionalization, like amines and other functional groups, and newer approaches and applications of such derivatives have been developed. So, here are a few references if you wish to know more about starch and pectins. And thank you, everyone, for your patient listening.