Matt Kirkegaard: Professor Michelle Colgrave, welcome to this Brewery Pro podcast.

Professor Michelle Colgrave: Thank you, thanks for having me.

Matt Kirkegaard: I guess the first thing, we want to talk a lot about gluten in beer and gluten intolerance and coeliac, but before we do we might start with telling a little bit about who you are and what your background is.

Professor Michelle Colgrave: I'm an analytical biochemist by training, so that means that I basically studied science at university. At first, I started looking at DNA and then I switched gears to start studying proteins.

Since then, I have been working for CSIRO for the last 13 years, I've also recently joined Edith Cowan University and my research group has been focused on detecting gluten specifically as a type of protein that's present in many foods and, of course, in beer.

Matt Kirkegaard: We're speaking to professional brewers who have a pretty good understanding of science, but obviously not necessarily to the level that you are. Let's step back and you just said gluten is a protein, but let's go back to basics. What is a protein?

Professor Michelle Colgrave: A protein is essentially a building block of life, really. What we have is we have DNA, which is our blueprint or the architect's drawing, and then our bodies make proteins but so do plants. In the case of wheat and barley and rye in particular they make these plant storage proteins, and these are the gluten proteins.

Gluten's this generic term that refers to these seed storage proteins that exist in these grains. They are there in the grain as a nutrient source for that plant to germinate. The brewers and the maltsters amongst this conversation will know that they rely on breakdown of protein during germination that occurs during the malting process and that is essentially the fuel source for some of these great flavour components. Another term we use is peptides, peptides is a small part, it's like a string, of amino acids that are related to the protein. So they're the small pieces of the protein that you end up with in your beer at the end of the day.

Proteins are these longer stretches of amino acids.

Matt Kirkegaard: If we're looking at, anyone that's been at a gym they've heard of proteins and things like that, gluten is a protein, but what makes it different from some of the proteins that you want, your meat proteins or some of the other proteins?

Professor Michelle Colgrave: Proteins fuel our body, and we have to get some of the amino acids from our food sources, so we can't make all the amino acids. We can intake other nutrients that contain carbon and nitrogen and other elements, we can make some of our amino acids, but we also have to ingest some of them. In our diets we're ingesting these proteins and then we're able to utilize those amino acids.

Now that's what we want normally from protein, but in the case of gluten they contain a different balance of amino acids, so they have a high proportion of glutamine and a high proportion of proline, and it's the combination of those that make them somewhat indigestible to some people.

Matt Kirkegaard: Why is that? How do the proteins break down in our body? What is the process there?

Professor Michelle Colgrave: When we ingest any food, we obviously chew it first and we produce saliva and that contains an enzyme called amylase. That usually breaks down some of the sugars. Then it goes down into our gut, then we have the action of a range of other enzymes, we also have acid in our stomach and so the acid and the pepsin, which is an enzyme also present in our stomach, starts to break down that protein. Then it passes through to our intestine, and in our intestine, we then have another suite of enzymes: trypsin, chymotrypsin, and these other enzymes, that then further work to break down that protein.

So the end result is we want to have amino acids that can pass across the lumen and go into our body and supply those amino acids for us to make new protein.

Matt Kirkegaard: What is it about somebody who has, we might speak about coeliac disease, is that the correct way to refer to it? Or is it just being a coeliac?

Professor Michelle Colgrave: So you could either say that they have coeliac disease or that they are a coeliac patient, either way is fine.

There is coeliac disease, which is an autoimmune disorder, and then there's some other aspects which are gluten intolerance, and that may be due to gluten. Sometimes it's the other proteins that are found in grains. But then there's also people who just don't feel well when they eat products that contain gluten.

Matt Kirkegaard: Let's talk about coeliac disease, someone who is in that category. What is it about them that gluten affects?

Professor Michelle Colgrave: As I was talking about digestion, firstly, because gluten contains these stretches of amino acids that are a bit more intolerant, well, indigestible. Normally a protein might break down to give just those amino acids on their own. In the case of gluten, it often, even in anyone, people without the disease, there will be stretches of these peptides, stretches of amino acids, that are not completely broken down.

In someone who doesn't have coeliac disease, that won't have any affect. That will just pass through the body and there's no trigger, as such. But those people who do have coeliac disease, their body will react to it. So there're antibodies that will come in and say, "This is not what I'm expecting, this is like a foreign thing in my body, and I'm going to trigger my immune system", so you go into a state of inflammation and then that can start, those antibodies actually start to damage the body themselves.

So you start seeing a thing called villous atrophy, which is basically when the lining of the intestine, they have these finger-like projections that are normally there to help absorb nutrients and they essentially get worn away. You can have a whole range of symptoms for people who are true coeliacs, and that can be anything from bloating to stomach cramping to nausea and a whole range of other symptoms.

Matt Kirkegaard: How about when you start moving into the - I guess as a spectrum of gluten intolerance you're talking about people who were intolerant to gluten - but then also there are a range of other proteins that are present in grain but it's not gluten being the culprit.

Professor Michelle Colgrave: There's a whole range of different proteins. I mentioned before about in your stomach, in your intestine, you have these natural enzymes that break down protein. There's also a class of proteins that exist in grains that are there as defence molecules against pests. Of course, the plant has to defend itself, it can't run away, so what it does is it has its own armoury. These proteins that it expresses are there to, the insect comes along, has a bit of chew on the leaf and gets a bit of a tummy ache itself. So it's not surprising that sometimes when we eat these plants that we might also get the similar symptoms.

There're these proteins called alpha amylase trypsin inhibitors, so they are proteins that inhibit the action of amylase, what's normally in your saliva, and it also inhibits the action of trypsin, which is in your intestine. So the job they do is by stopping those enzymes from breaking the proteins down, that can actually mean that you don't have full degradation of proteins as well.

Matt Kirkegaard: And what does that do to the body?

Professor Michelle Colgrave: So again, this can lead to similar symptoms that you might see in coeliac disease, you might have some of those gastro-intestinal distress symptoms, but they are not necessarily causing the same affliction that you get with coeliac disease.

Before we move on to some of the non-gluten grains, there are grains that brewers use to make gluten free beers, are some of those other proteins in them? So, for example, if you were able to have a gluten free beer for the gluten, would some of those proteins still crop up in a gluten free...?

Yeah, those classes of proteins are generally what we call "ubiquitous", so present across many different plant sources. They are different in some, and sometimes it's the combination of different proteins together that will cause a reaction. I think there is a definite link between the amount of these other proteins in the same grains as you get gluten from. What they typically termed it is non-coeliac gluten sensitivity, so still the same grain types but maybe different proteins or different things that are triggering similar symptoms.

Matt Kirkegaard: Okay, so someone who is in that category of intolerance would still be able to have a gluten free beer, we'll explain what a gluten free beer is. So they would be able to have a beer that's made without barley or wheat or some of those and probably not suffer those other symptoms?

Professor Michelle Colgrave: I'm not gonna give a definitive answer on that one, but it is possible and probable. But there could still be lesser amounts of them, but they may not have as acute of symptoms, for instance. There hasn't been enough research to fully understand how other grains may have similar type effects.

Matt Kirkegaard: Let's talk about gluten free and gluten reduced.

We've seen a number of brewers who've started making beers with non-traditional brewing grains that are gluten free, they're able to label their beer gluten free, is that correct?

Professor Michelle Colgrave: That's right. Before we get into which is gluten free and gluten reduced, what I'd probably define is what does gluten free mean?

Matt Kirkegaard: Absolutely. Does that change from Australia, North America, and Europe?

Professor Michelle Colgrave: I was exactly gonna go there too.

So if you're in North American or you're in Europe, it's a little bit clearer in that gluten free is termed as the 20 part-per-million, or 20 milligrams per kilo threshold, so that means in every million molecules, essentially, only 20 of them can be gluten. That's the limit that's been set according to the clinical studies that've been undertaken and that's generally deemed as safe. You just need to be below that.

Matt Kirkegaard: At that 20 parts-per-million, the body's able to process that? Or it's not going to trigger people that are gluten intolerant?

Professor Michelle Colgrave: It's generally set for 99% of people to be safe. There's always going to be someone who's more sensitive and may still get a response, but it's generally, they try to set it at that 99% safety level.

Now, you've got the threshold that's been set based on clinical relevance and the reason that's important is because there's always going to be a small amount of these grains entering food systems just because they're grown together, they may be transported in similar truck, or they may be manufactured in facilities. There's always a small amount, but it needs to be a very small amount. It's gotta be clinically relevant but also practical.

In most jurisdictions 20 parts-per-million is the level. In Australia, gluten free means that it contains no glutens. That means that it can't contain wheat, barley, or rye, so we essentially have a zero threshold, so no detectable gluten.

Matt Kirkegaard: Is that a good thing or a bad thing?

Professor Michelle Colgrave: There's pros and cons.

It is a good thing for, I guess, from the perspective of if it contains any detectable gluten then it cannot be labelled in that way and that means that it's going to be safe for someone who's a true coeliac, but then the problem with that is that you could have this small incidence of very low levels that aren't going to be harmful to most and it's harder for manufacturers to adhere to that very strict limit, and thereby product availability might not be as great. So there's kind of two sides to that story.

Matt Kirkegaard: What does gluten reduced mean then?

Professor Michelle Colgrave: Gluten reduced is, there's also another term that they call low-level gluten.

Matt Kirkegaard: Is this in Australia?

Professor Michelle Colgrave: There's different jurisdictions as well, and this might set at 100 parts-per-million or it might be set at 200, so there's different levels that are applied and this is where some of the confusion comes in.

Gluten reduces means that you've started with a material that contains, I'll just talk about beer for the moment, barley in this case. But what they've done is applied a treatment of some sort to remove that barley gluten. In many instances you can make a product, for instance in wheat you can make a wheat starch where you can purify the starch out removing all of the protein, and you can end up with a starch that's actually completely free of gluten and therefore will be completely safe for anyone.

But in the case of gluten reduced, as they apply it to beer, typically they may add an enzyme and that enzyme is added during the brewing stage and that is either to degrade the gluten. There are other enzymes they use that are actually there to transform the gluten into a different form, and then they can sometimes filter it out.

So that's the process. It's a means of reducing the gluten from the level that it would be.

Matt Kirkegaard: It breaks the gluten protein down, but it doesn't remove it, is that correct? Or degrades it in a way that it doesn't affect the body? What's the outcome there? So it's reduced in terms of its testable presence, but is it still there in a form that can affect the body?

Professor Michelle Colgrave: I might go back to the brewing process for a moment and just talk about what happens to gluten during a routine brewing process.

Gluten is automatically reduced during the brewing process. Often it will precipitate and so then can be removed during different stages or it can cross-link and that crosslinking will cause it to precipitate, and some of it's degraded by the natural enzymes that are present within barley grains. These proteases that exist, so when I talk about protease, I mean an enzyme, so I give them interchangeably. Their job is to break down proteins, so some of it is broken down just during the course of brewing.

Then during, when they add these enzymes, the role of the enzyme is actually to break up the gluten proteins and its targeted specifically at gluten because these enzymes are typically what we call a prolyl endopeptidase, and that's a long work for an enzyme that cuts up prolines and, if you remember back to the start, I said the gluten was full of proline and so its job is to break up these prolines and therefore remove or shrink down the size of the protein. Chop it up into lots of pieces. So if you imagine it as a long string of amino acids, that it's gonna chop at each of these prolines and you should end up with smaller pieces that are cut at those site.

Now in many cases that might be enough to reduce the level to what might be safe, but in other instances it won't be safe because there's still a level there that people can respond to.

Matt Kirkegaard: And when you say instances are we talking about particular people and their susceptibility to it? Or is it if it's not used correctly? What's an instance?

Professor Michelle Colgrave: Little bit of both there. With any of these diseases there's a spectrum of sensitivity in the population, so while one person might not be able to have any symptoms at all, the next person will have a severe reaction, and that's due to the sensitivity to the particular proteins.

So there's that, but then you've also got the combination of if you don't apply the enzyme in a way that will cut all of the gluten, then it's going to remain. And what the literature and the scientific studies have shown so far is that there is a significant amount of gluten that remains just in sizes and forms that can't really be detected by the current standard methods.

Matt Kirkegaard: Which brings us to the next thing. We've got this process, but then testing is a whole other kettle of proteins, for want of a better word. So how do we test for gluten in something like beer?

The current method for testing for gluten is what we call an ELISA, and ELISA stands for an enzyme-linked immunosorbent assay. It basically makes use of an antibody. We make an antibody, scientifically, essentially, we make up these antibodies that will react specifically to different types of proteins, in this case gluten.

In a standard food where there's not been any fermentation, especially in grains that are whole, then we can use what we call a sandwich ELISA and that's a very straightforward technique, and it works really well in wheat where you have wheat as the standard that they use to calibrate this assay and they can detect wheat really well.

It becomes a little bit more challenging when you switch gears from wheat to barley because it gives a different response, but that still can be accounted for. But when we start dealing with beer or any product that's been fermented what I said already is that during brewing you have this breakdown that's occurring anyway, so you've already got these pieces of protein that aren't intact anymore.

The antibody is like a lock-and-key mechanism, so essentially the piece of gluten needs to be the key that you insert into the lock in order to unlock that and get answer. So with that, if your key doesn't quite fit the lock then you're not going to get the result. What's happened is you've got this breakdown of the protein already and so it's kind of changed the shape of the key, and then you're applying this enzyme and then it might, again, change the shape of the key a bit more and it just not be able to unlock and get an answer.

In many cases you will have a fragment that can't be detected by this antibody anymore because the antibodies specific to a particular site on the protein. But the rest of the protein might be there, it just becomes invisible. Another thing that can occur -

Matt Kirkegaard: Effectively invisible as opposed to not being there.

Professor Michelle Colgrave: That's right. It's invisible to the test.

And another thing that can happen is that you have all of these other great things going on during the process, so we know that we, during malting there's a kilning step, there's glycation that occurs which is essentially this sugar-based reaction. Some of those sugars can end up on the proteins as well, and if they do they can essentially hide that site that the antibody would normally bind to.

Matt Kirkegaard: And I guess for all of these ways that protein can be hidden in testing, it's not hidden from our body if it's present in the liquid.

Professor Michelle Colgrave: That's right. Sometimes that breakdown will be sufficient to hide it from the body and not have those reactions, but what we think is that, for the most part, the antigen site, this is the part that the antibody binds to, they key as such, will have been removed or decreased but the rest of the pieces of gluten still exist.

That takes me to my research, where we've been using an alternative technique and that's another long name, liquid chromatography mass spectrometry, or LCMS, so I'll use LCMS for short. It's a different technique altogether, so instead of having to rely on an antibody to see it, we just use this mass spectrometer to directly measure that protein, or those peptide fragments. We take the beers and we do a little bit of treatment but then essentially apply them to this technique and then we can directly measure what is present. We measure the peptides, these small pieces that still exist, and then we also look at some of the proteins as well.

Matt Kirkegaard: How expensive is that process?

Professor Michelle Colgrave: The process, well, it's more about the infrastructure, I guess. You're not gonna have a brewer who's gonna have a mass spectrometer in their garage, it's just not gonna happen.

Matt Kirkegaard: And I presume that, because of the process to make low gluten beers, or gluten reduced beers, you would need to test every batch.

Professor Michelle Colgrave: You do, and that's one of the things we've seen is that when we use this new technique to look at batch to batch variation, it's really variable. Because even though these enzymes cut at these prolines, they're really not as specific as some of the other enzymes we use for science purposes. So you get a lot of variation, and you also get a lot of variation just through the brewing process.

With all that variability you absolutely have to measure batch to batch, and you may use a different batch of the enzyme this time or you've used it for a slightly longer time, or perhaps the temperature was different.

Matt Kirkegaard: And that's the interesting thing is that these enzymes that brewers use to create gluten reduced beers weren't developed to reduce gluten, they were for a range of aesthetic uses in the brew house.

That's right. One of the enzymes was initially introduced because they wanted to remove haze. Now haze is quite trendy these days, but it wasn't always.

Matt Kirkegaard: Remove the proteins or denature the proteins that created the haze. The by-product was that those proteins-

Professor Michelle Colgrave: So what happens is the haze is actually the reaction between proteins and polyphenols, so these two come together and they form the haze that you would see. If you can break down the protein, it also breaks down these protein polyphenol complexes and thereby clarifies your beer. So that's how they were initially utilized, but they hadn't really been optimized to specifically break down gluten. It was more of a side finding and application.

The challenge is that, when you're using these techniques that's great if you've got a tool that will effectively measure how well you've applied that enzyme and making sure that, once you've applied the enzyme, that there is really a reduction in gluten. And you might be reducing the intact gluten, but you can't measure, with the current test, whether the small pieces that remain, you just can't see them. And what we still don't know is, these small pieces that remain, we don't know exactly how toxic they are because there are so many of them and they're all so different.

Matt Kirkegaard: And by toxic you mean inflammatory or affecting people that are intolerant to them.

Professor Michelle Colgrave: Yeah, that's right. But we've got anecdotal evidence. Well, sorry, I should say that there's also evidence coming out of some of the researchers in the US who have taken, essentially, the T cells from people who have coeliac disease and they've applied these gluten reduced beers to say does this stimulate a T cell response? So this is essentially, it's a lab-based method to say, "Will this likely induce the same response in a patient?" And in some cases, it does.

But we also have explored the peptides themselves and looked at them to say, well we know which part of the protein, theoretically would create a response, and we've seen those parts still present in beers.

Matt Kirkegaard: At the end of the day, what is your recommendation to beer drinkers as expressed to brewers? How should they be labelling beers that maybe use traditional grains, but they've treated to reduce the gluten? How should they be communicating?

Professor Michelle Colgrave: You will have seen that in the US they've moved to a position where they use things like "crafted to remove gluten" or "gluten reduced"-

Matt Kirkegaard: That's a marketing term as opposed to a scientific term.

Professor Michelle Colgrave: These are at least provide some transparency in that they're not saying that they're gluten free, what they're saying is that they started with gluten they have treated them in a means to try to reduce

the gluten. And I think that that is the minimum requirement we need, because you need for it to be clear to the consumer that there is a possible risk.

If they decide to try them, they should do that under the guidance of their medical practitioner so that they can monitor the long term health effects. Because even though someone could drink a beer or two and perhaps not get acute symptoms, we don't know what the long-term chronic effects could be. So it really needs to be done under the guidance of your medical practitioner.

Matt Kirkegaard: And how about gluten free?

Professor Michelle Colgrave: Gluten free, if they are endogenously gluten free, that means that they are made from things like millet or sorghum or oats and quinoa, there's a whole range of different grains that have been used to make gluten free beers, and in those cases they should be gluten free simply because they don't contain barley, they don't contain wheat, they don't contain rye. So they should be no risk to consumers.

Matt Kirkegaard: You're also involved in a couple of projects to breed low gluten strains of traditional brewing grains, how is that research coming along?

Professor Michelle Colgrave: I was really fortunate to work with a group of scientists at CSIRO who took a different approach to this and said, "Okay could we actually remove the gluten from the grain itself?" What they did was they looked around the world to see what varieties existed and then they worked on barley.

In the first instance they found, there's four classes of gluten that exist in barley, and they're simply called B, C, D and should be G, but they call it Gamma because they're scientists, but they found a variety that didn't contain the B-14s, which is one class, then they also found a variety that didn't have the C, so they crossed those to create what we call a double-null, and then they crossed it again with this D-null and so they essentially eliminated something like 98% of the gluten that would normally be in barley.

Then what we did was, we used this mass spectrometry technique to ensure that the grain that was being produced actually really did not have the gluten there, to confirm that. And we also used it along the breeding process, because this is not genetically modified, this is not gene-edited or any of these other techniques, it's just a selective breeding program. Taking one grain and crossing it with another. And so we were able to produce this barley, it's now known as Kebari, and it is gluten free according to the US and European standards. But not by the Australian standards because it contains barley.

Matt Kirkegaard: Do we need to work on the food standards to be able to use it here, or do we need to come up with a whole new-

Professor Michelle Colgrave: There has been some review of what the gluten free standard should look like in Australia, and that review's ongoing. We may see that that is changed in time to come in line with what we see overseas. In the meantime, I guess, it's still possible to use this grain in brewing, it's just a matter of how you market it.

And we have got a couple of examples. I think Bentspoke in Canberra were one of the brewers who actually went in and tried the Kebari grain and produced a really great beer using it.

Matt Kirkegaard: I'll follow them up to report back on that.

Has there been any testing done about how it works in the brew house, if there's any difference to the other components of the grain?

Professor Michelle Colgrave: One of the initial challenges in the first crosses is once we removed these proteins, and we're removing up to 50% of the grain protein, was that the grain itself was smaller and it had this shrunken, sort of like a hollow, so instead of a smooth plump grain it was actually hollowed out, and that meant that it initially created some problems with filtration.

Through a series of additional breeding lines, they managed to increase the grain size back up to a point where it didn't create those problems anymore. That was seen as an early industry issue and they managed to fix it over the coming crosses.

Matt Kirkegaard: For all of your many expertise areas, brewing may not be one of them. How was, with protein being important to, for example, head retention in a lot of beers, how did the head retention get affected?

Professor Michelle Colgrave: That's something that I do get to test a little. My research groups actually don't mind this part of the job because we do open many beers in the lab.

Matt Kirkegaard: You are a beer drinker.

Professor Michelle Colgrave: I am a beer drinker, and we do open many beers in the lab, and we do sit there and have a look at some of these things because they all do perform very differently.

One thing is it does pour quite well, and you do get the head but it's not retained for as long. So you do start to see some of those things, but if you're, I guess someone with coeliac disease or a gluten intolerance, I guess head retention is not your biggest issue.

Matt Kirkegaard: The number of people that are just desperate, they love beer and they miss out. How about cost? How is cost affected with Kebari? Is it being grown at a scale that the cost is relatively comparable to traditional barley?

Professor Michelle Colgrave: I don't have the numbers off the top of my head, but it would be grown as a premium because you do have to make sure that you're keeping the other barley out. With that you have to have closed-loop growing and contract growing so it's not going to be directly comparable but, again, these are products that typically fetch somewhat of a premium because of the fact that you have to do the additional testing. There are a number of steps along. Any of these free-from foods, where there is a requirement for additional testing and to ensure product safety, so there will be a slight cost increase.

Matt Kirkegaard: This is one of those areas where we could talk for hours, but we might bring this particular class to a close.

Is there anything else that you think that brewers need to know about gluten free and gluten reduced within the context of this conversation?

Professor Michelle Colgrave: I think it's just all about providing as much information to the consumer as possible, and that's where what they label the products as is really important. You need to be able to understand that, yep, they've used an enzyme here. I don't quite understand what an enzyme is, perhaps, but it started with barley in it and they've taken their best effort and they've used the testing that's available. We hope that the types of testing will improve over time and be made available to everyone, but at this time I think it's about providing transparency and labelling.

Matt Kirkegaard: And that brewers should understand the limits of what, when they read test results, what those test results are actually telling them.

Professor Michelle Colgrave: That's right. If you know that you've got a means of measurement that is going to be accurate and reliable, that's fine, but we do know that there are some challenges to the current means of testing and that information probably isn't passed along to brewers. I don't know how many brewers are avid readers of scientific literature, but certainly we've been trying to disseminate that information broadly because it is a challenge.

Matt Kirkegaard: Wonderful. Professor Michelle Colgrave, thank you so much for joining us on this Brewery Pro podcast, and we look forward to following your research and some of your other studies very closely.

Professor Michelle Colgrave: Well thanks for having me.