Good morning, dear colleagues, friends, and family. Welcome to my PhD thesis defense. The title of my work is Characterization of Apple Pectin Structure in Relation to Apple Fruit Processibility. In the next few minutes, I would like to introduce what I have done during the past few years. As we all know, the apple is one of the most popular fruits in the world. And the production is nearly 100 million tons per year, and beyond the huge number of production, there are over hundreds of different cultures agreeing, and each offering a unique appearance of flavors and textures. Besides the fresh consumption, the huge numbers of the apple are processed into juice and the puree. For this liquid and semi-liquid product, the quality depends on several indicators. However, the major defect is the cloud separation. This issue is related to apple's structure, especially its pectin. So here we go to the central molecule, the pectin. Pectin are important in food science in plant. It's a fundamental cell wall material that holds cells together. And the second, pectin is a key for the ingredient. And third, the pectin provides the health benefits as a source of dietary fiber. Pectin is not a uniform molecule. Its structure is complex and varied depending on different factors, like the source, the enzyme activity, the chemical emicab, including the sugar composition, the DM, and the method of how they extract it. And one of the structural features I found out is the methyl ester distribution of the methyl esters over the pectin backbone. So this figure shows why it's important. As we can see, even for the pectin with the same DM, the methyl ester distribution can be varied among the different pectins. So we know that the apple product is related to the apple's pectin structure, like the DM. So this will answer a question that if the methyl ester distributions can also affect the product's quality. So this leads us to three research questions for this thesis. So first, we want to know what are the pectin structure's differences among different cultures? And the second, how do these structural features affect the product's quality? And finally, what are the key metabolic factors affect these structural differences? So to answer this, we set up three parts workflow with one main method. First, we extracted two types of pectin, the water-soluble pectin, and the chaletra-soluble pectin, also called the calcium-bonding pectin. We then characterized them in details with the sugar composition, degree of methyl esterification, and most importantly, the methyl ester distribution. And then we processed the same apples into product and measured the quality of the product. And then we used the statistic method to link the product quality to the pectin structures. And then we used the metabolomics approach to find out which metabolites may affect the pectin structures. So the main method we use is the intermetric fingerprinting. So we use two different enzymes, one PGA and the PL, to cut the pectin backbones to fragments. And from the fragments profile, we can calculate some descriptive parameters to describe the distribution of the methyl esters. This give us molecular level insight of the pectin structures beyond just the DM. And here are some results. First, we looked at pectin from certain cultivars. The water-soluble pectins are all highly methyl esterified, with some slightly differences in the sugar content. While the chaletra-soluble pectin are more linear and with a higher content of glycolonic acid, with diversity in their DM, some cultivars had a high DM and some cultivars had a low. The intermetric fingerprinting shows that even for the same pectin type, different cultivars can give different fragment profiles. So two pectins may look similar in DM, but their methyl ether distributions can tell a different story. Next, we checked the juice quality from the same cultivars. We found the juice quality varied a lot among the apple cultivars. Linking the product's quality to pectin structure, we found that the pectin structure features, especially the methyl esterite distributions, were strongly related to the juice quality. So it's not just the level, but the distribution patterns of methyl esters are also important. Similar to apple juice, our purists from different cultivars show the distinct textures and flow behavior. Correlating results showed that the methyl ester distribution parameters were significantly linked to the rheological parameters, properties of the puri. And this confirms that the detailed pectin structure does not just influence the liquid product like juice, but also shape the texture of more complex product like puri. So we knew the pectin structure differs

by cultivars. Another question is why do these differences exist? To find it out, we use the methyl bollomics. We found 11 classes and 17 subclasses of metabolites that differ across cultivars. And using the VIP scores, we identified which metabolites were most responsible for the differences between cultivars. And this gave us a list of candidate metabolites to study further. With that list, we used a different metabolic tooth to figure out which metabolites are linked to the pectin structure, to certain pectin structures. For instance, the neutral sugar content, the DM or the methyl ester distributions. And we also want to know how stable the pectin structure is from year to year. We compared the eight cultivars from two years, the score plot showed how the cultivars grouped based on the pectin structure across year. And the VIP scores showed which parameters cost this year to year differences. And we found that the neutral sugar are stable, but the methyl esterification changed a lot. Why? It could be the environmental reasons, some wider or the growing condition is different or it could be enzymatic. The enzyme activity may vary by years and the change in the methyl esters. We do not have the full answer yet, but this gave us a direction of further study. So let me summarize what we have learned from this thesis. First, we found some significant differences in the IPO pectin structures among different cultivars. And the second, even for the same IPO cultivars, the different harvest year can give the pectin structure in different like the DM and the methyl ester distributions. And the third, the native pectin structure can significantly affect the quality of the product. And the fourth, some certain metabolites can influence the structure features of the pectin. So looking forward, our future research should systematically monitor pectin structure and with different omics studies and with small IPO cultivars included and considering different factors to achieve the more comprehensive analysis. Before I finish, I have some people to thank to my supervisors, thanks for your mentorship and the support and to my co-authors. Thanks for helping me to see my work in different view. And to my colleagues in FCH and CAS, thank you for creating such lovely environment and to my panelists, thanks for your preparation for today when I was not in the Netherlands. So here's the end of my talk, finally. Shortly, the opponent will enter and the 45 minutes public defense will begin. And after that, the committee will leave the room for their consultation, then if everything goes well, they will come back and I will be awarded the degree. After the ceremony, there is a reception outside and you are all welcomed. Thank you. Please be seated. I hereby open this ceremony convened by the Academic Board of Wageningen University in which Darcy Liu is offered the opportunity of defending a thesis with propositions entitled Characterization of Apple Pectin Structure in Relation to Apple Fruit Processability. The defense will take place before an examining committee appointed by the Academic Board as a prerequisite for conferring the degree of doctor. Good morning, I'd like to welcome you all to this graduation. My name is Tini van Buckelen, Professor of Food Quality and Design and representing the Academic Board and Director of Magnificus today. I now call upon the first examiner, Professor Fogliano, who is Professor of Food Quality and Design at Wageningen University. The floor is yours. Thank you, Mr.

Rector, respected candidates. It was a pleasure to read your thesis, very interesting topic for me and I would like to start your position, asking you to ask one of the parnif to read the proposition number two. Hi ladies and gentlemen, it's been the opponent's second floor candidate, their parnif, can you read the proposition? The huge variation in compounds in an apple exceeds the wide range of apple appearances. Can I, basically I philosophically disagree on this, but I want to know why you, what's your take on this proposition? Yeah, let's say when we go to the supermarket, if we can find different apples, like the yellow one, the green one, the red one, and so let's say the difference is directly there, so we can find it easily, but inside the molecules there are different human, when the apples are the same. Looks similar, the difference there should, there will be some differences in between, I should say. Yeah, but don't you think that we as a chemist, I'm a chemist too, but I mean, we are so obsessed by little variation in the structure that we think they make a big diversity, but actually the diversity

of the nature, like having a dozens of apple variety, that's what matters. It's just a small clever that we are interested in this level of details, but actually for most of the people is the variety of nature that matters. Yes, so let's say that when we look at, I say the packing our molecules, we can find that even for the same cultures, we can find differences between, so we cannot really say just from the overview of the ourselves. Yeah, but just to make a translation in humans, me and you, we are different. We are different, we like that we are all different in this room, we are made the same. Proteins, carbohydrates, glycogen. Yeah, of course there are difference in the proteins, I mean, some amino acids are different, and so chemically we are different, but what it matters is that we are different as individuals. So that's, I think we have to be careful, that's my point in over-exaggerated the relevance of chemistry diversity. But okay, that's nice to start from philosophical part, but let's go into the apple, and I would like to understand better, yeah, if you had to explain how packing structure change the physical characteristic, the sensory characteristics of the, for the consumer experience, which are the relevant, how the packing change the sensory experience? Actually, it's a quite subjective choice. Somebody may like the sore one, maybe somebody like the sweeter one, so we cannot give an exact answer that's which one is most like, we can only do collecting some more data to find the most important, I'd say that the flavor of the. Yeah, but other other parameter like turbidity, or these are, you know, something that we can objectively measurements. Also the sensory can be objective, but okay, that's a physical parameter can be objectively measured, how they are influenced by the packing structure. Like I say, for example, the turbidity, the turbidity is related to the particles inside of the product, and some packing has some charges, and they will show on the, by the, let's say the protein, and they will settle down, and if we call it cloud separation, and if we found the separation of the clouds and the juice and the liquid, it will influence the appearance. So it may affect the consumer's acceptance of the choice, but somebody may like it, but I think it's more nature, but other people think we will go by that or something. Yeah, but you keep on saying this subjectivity, but if I look at the figure three on page 132 of your thesis, I guess there is a heat map correlation of the chemical composition and the physical properties. There, I mean, it seems like red, yellow, so there are some parameters that are very influential and some other not. So it seems that there is a kind of a one-to-one correlation. Yeah, it's only a correlation, but not, we cannot say it's like a cultural relations. It's, that's not some parameters cause that defect. It's a system, we think food is a system, so we cannot, we can only identify which parameters or which structure features are related. We cannot really tell is the reason that caused this phenomenon or the physical. That's, now I've become a little bit uncertain of, because I made you a very simple question. I like the pink lady is one of your varieties that you study, okay? So if I want to know which are the pink lady characteristics in terms of pectin mainly that make the pink lady the food that I like over the other. Is the composition of the pectin, is the degree of methylation, is the branching, there is something that we can correlate to the, and pink lady is a very peculiar variety, very crunchy, so what is the, that make the difference? I should say this is the, it's co-factors influencing, but we cannot tell only one thing's effect, I say the properties. Because I was surprised that you report that the pectin metal esterase, PME activities, the pink lady has the highest among the 13 varieties that you studied. So I thought, oh, now this is why. There is a very high pectin metal esterase activity, so maybe the de-medilation is very high, so this is not possible to correlate. Yeah, it's like our research is kind of explore with research, so we just want to know if there will be a correlation, and in the future study may look in more details to. I think then I have to say that you overstate in your thesis, because for instance, in the page 108 is the abstract of chapter four, and you conclude the abstract, say these studies offer insight for utilizing plant polysaccharides diversity in food design. So the way I read this is that you have the ability to decide, okay, I use these polysaccharides to have these properties in the product. Yeah, it's like I said, the pectin is the fundamental molecules in

the, I say the juice, the products, so only if we understand what's the pectin structures, so I say it's the very first step. It's a big word here, but yeah, it's our dream to our final goal to achieve this, so we have to start at the first step. I don't think, I'm not sure this is the case. If I go on a catalogs of ingredient providers, they actually provide you different pectins with different chemical features for some applications. So there are some things that we know in terms of properties of pectin and use in food design. Yeah, but our pectin is from our, I put the material, the raw material, and it's a blueprint, I should say. We cannot avoid our molecules inside of the food already there, so. Yeah, yeah, you are studying how the pectins are in food, but you can extract the pectins, right? You have a pectin extract from apple, and you can define for this extract with a certain composition, certain properties, and that's what I understood was the idea from your thesis, and all these questions are about, tell me some correlation possible. I'm not saying that it's one-to-one, but some trends. Yeah, it's just like we are trying to find the trends that which one affect which one. And give me one trends. Yes, the, let's say the degree of, the distribution of the mesoesterification if we have longer blocks of the mesoesterification part, it will give some charge to the molecules, and it will easier, I say, to aggregate and to settle down. Okay, thank you. I give back the floor to Mr. Rector. Thank you, Professor Focleano. The opposition will now be continued by Professor Quimbera, who is Professor of Chemistry at the Department of Chemistry at the University of Averro in Portugal. The floor is yours. Thank you very much, Mr. Director. Respected candidates, can your perinames read preposition number five? Okay, dear Periniff, could you read out the proposition number five? Independence within a collective is highly relevant, but for an individual, it is meaningless. Thank you very much. Can this preposition be related with position number two? That was already read, and say, explaining that, okay, there is some independence within a collective, that independence is highly relevant, but it's not meaningless. And this is the purpose of your thesis. So this can be the conclusion of also your thesis. So the apple, by itself, contains the same compounds as individual, is not really meaningful, but it can be to distinguish from the other type of individuals. Can you explain on that? Yes, at first, I saw this proposition as a human, not a real productive. So this level, I didn't mind, but let me think. I know you were talking about humans. Yes, but you are also, you are mixing also the plants with the domestication in the other propositions with, by the humans. So this is kind of individuals are really important, depending on the level we are looking to the things. Yes, but let's say it's a dialectical question that's how do we define the individual or the collective, and yeah, for, I say for a single apple, I use the apple as an example, for the single apple it could be sweet, but combined, I'd say many apples, the flavor will change. So independent here is not the... Like individuals, like individuals. So we are all different, but we are also made of all of the same molecules, like the apples. So it's how do you, I could use the frame of the reference, so how do you judge which one is individual, if we look one molecule, it could be individual, but if we look a single person, it also could be individual. So it's a question of us. Okay, I would like also now looking for the details, in fact, there are some details, and then one of the details, so you are talking about and you are studying pectines, but you are extracting with hot water and you are extracting with the collating agents, and so you have in some of your samples, I'm now looking again for the page 132, for the figure three, just the same as Professor Foglien was looking, but now for looking for another aspect, that is the presence of glucose in your water extract material. So can these glucose come from starch? Can this influence the turbidity? It could be a trace amount that's from starch, but it could also be from some cellulose or a bit. If it is so, if it is insoluble, yes, but using our extraction method will already exclude some starch, some, I say the cellulose, they were remaining in the residues. Yeah, but starch can be extracted with hot water, with some amount, some amount of the extrins and can provide some properties together with the pectines that you are trying to relate. That's my question. Yeah, it could be from starch, but also that the starch amount in the apple is not that high, so we just- 5%. So

we overlook this, and also with our extraction method, there will be even less, so we did not take this glucose out, stashing to consider. Okay, so when you have, so you did the extractions of the pectines with high temperature. Usually, so to have pectines that are in the native form, so that sometimes is very difficult to extract. We avoided use of these high temperatures. So in this case, it seems that you may be degrading some of the side chains of the RG1, yes, the hot water, but first of all, it's already the, like I say, the common method. We cannot really avoid to break the pectine structures, so we use this, it's already the mild one, mild method we can use. And yes, it could be destroyed the structure a bit, but we checked with our HP psych and still the, let's say the molecular weight is there and over the 100 kilo, so we think he's doing things. It's always a compromise. Yeah, we cannot really avoid to, of course. You need to apply chemistry, so it's a compromise between not to be so destructive and applying the same procedure to all your samples in order to have this type of comparisons. Also, you establish a kind of parameter that is the RG1 side chains. And you assume that, so all, you assume that the ramnose is, so you compare the amount of arabinose and galactose to the ratio of ramnose. So you are assuming that all ramnose can contribute to the branching points. But in fact, you have some ramnose that is not involved in linkages with these neutral sugars. Can you, or could, probably you didn't have time, of course, because you need, again, it's a compromise. But if you have time, what is the procedure that you think you could do in order to evaluate if you have this, to exclude that ramnose that is not involved in the branching points? Yes, the ramnose are there, and we cannot say that it's from RG1. But I think we cannot really exclude the RG1 because we try to understand what's the native packing structure. So maybe in the future work we should, or we, I say, use a different method to find where the ramnose come from, and how more accurate the description here is. For example, doing a metallation analysis, you can find what are the amount of ramnose that is one to four linked, or is one to linked. And based on this difference, you can add another parameter, that is the length of the ramnose that is substituted. Yeah. Yeah, we just, let's say, at first we want to understand only the metallister distributions, and we found that there are still some sugars inside. So we, maybe in the future work, we will take it into consideration and use some different, let's say, the MRO. Yes, because, so at this is only to pave the way for other studies that could appear. So in your case, so I think you have a lot of this analysis, so you have lots of analysis, but it shows us, highlight us some points that we could explore in the future. So another final remark is related with some structures. So working with carbohydrates is not easy. So, and it's quite easy to have some, some errors in the structures that we are doing. So I would like you to look at your structures that you have, let me see where is the, where are the structures? I put it here, oh yes. So it's in page five and page 10. So the structure in page five, if you look at this, so I don't know if you remove this from the internet or if you leave that. So, but that galacturonic acid that you wrote is not galactose, but it's glucuronic acid because it should be, the linkage should be axial, not equatorial. So it's only a small detail, but it's quite easy because the structure is very difficult just to identify and to write. So, and another thing is related with figure three in page 10, that one of the OHs that you painted in blue, it was good to put this OH in blue, this cycloboxyl in blue, because just to chelate it with calcium, but in fact, the direction of the OH should be the other one. Okay, so this, I should say, to be honest, this is from the literature research. Yes, so it's always, so I'm liking that because I'm a chemist also. And that is the point, so to pay attention that sometimes the structures we are writing sometimes are not correct. And this is pedagogical, so search for the other ones that can replicate the errors if we keep doing this type of error. So, Mr.

Director, thank you very much. Well, thank you, Professor Quimber. The opposition will now be continued by Professor van Louis who is professor of food and microbial technology at the Cau in Belgium. The floor is yours. Thank you for the nice introduction. Respected candidate, I've read your PhD thesis with great interest,

and thank you for your contribution to science. My first question goes on the link between chapter two and three. So in chapter two, you extract certain pectin fraction and you do a detailed structural characterization. In chapter three, then you produce apple juice and you determine certain physical chemical characteristics. And then you do a correlation study by multivariate data analysis to see which structural characteristics is correlated to certain physical chemical property. My question is, could you not formulate upfront already in a hypothesis and based on certain mechanisms of which correlations you would expect? Ali, it's an opponent. Thank you for your next word and also for your questions. Yes, at first, we have the hypothesis that the massive distribution is related to the tubidity and the stability because we found that the apple juice will separate over time. And we also think that the data potential is related to the, let's say, the colloidal stability. So we have this hypothesis and then we also found that it did related to our massive distribution parameters. So it's, but it's only our first step because we know the food system is complex and we cannot really say the massive distribution cause this aggregation or separation, but it's only our first fund. Yeah, let me go into your answer a bit. So you say with zeta potential and zeta potential is then linked to indeed the distribution of charges over your pectin chain, but are you not neglecting some endogenous compounds in the apple that might influence the zeta potential and that you did not characterize? Yeah, so there are some, I say the organic acid or some other component, compound will influence the, let's say when we married the zeta potential, we let's say dilute the samples with buffers. So we try to avoid this kind of interference. So, but we cannot really, I think it's a system. So we cannot really avoid this kind of interference. But in the juice, I'm more referring to there is endogenous cations like calcium ions that might shield your charge distribution over the pectin chains. So yeah, that is interfering in the relation between the structural characteristics and the juice. How would you in future take that into account? Yeah, it's a tough one because let's say we, as I mentioned in my speech and in the future, we should like monitor all the things because we, at first, we think that's only the matter-user kind of fact, but we may really look into the messages and we found this much more work we should do. So maybe- David, can you give me some specific experiments that you would carry out to also include, for example, the role of endogenous calcium ions? The most simple one is to mirror the content of the calcium and try to know the concentration inside. So I don't know, I can only think about this but I will think about it in the future, so. Yeah, another question related to that correlation study is that now you have determined the physical chemical properties immediately after juice making. So I have multiple questions on that. How did you prevent that there were further enzymatic activities while measuring because you didn't inactivate enzymes upon juicing? And secondly, in a practical application, there is always a preservation step, either a pasteurization step or something that also might change the physical chemical properties. You did not include in your work, so can you elaborate a bit what you think would be the implications of an extra preservation step? Would the correlation still hold or not? Yeah, for the anti-oxidation, we add some vitamin Cs to prevent the browning to block the activity of the PPO. And yeah, we did. But they are not working on the pectin, so I was not worried about the PPO activity but more about, yeah, pectinases that are still present and that might change your properties. So we do the other measurements immediately and not took it too long. We cannot really avoid this enzymatic activity or enzymatic actions. So because we think if we use some heat, they will change the properties of, maybe change the properties of the pectins and they will include another factor, so it will be more complex. And then the impact of the preservation step? We didn't think about it. In the industry, there should be some heating. In our, I say it's a fundamental experiment. So first, we want to know how the pectin affect the product's quality. And then if we have a direct direction or answer, we then will consider about it. Yeah, I do understand you could not investigate also. You make certain choices and I also fully respect, but my question is, can you, based on your insights that you have gathered, do some hypotheses on what would be the impact of the preservation

step? Yeah, I say the most common measure to use is the heating. They will certainly, because our truth is at low pH and some certain, some good degradation, the better elimination will happen. So maybe the molecular size of the pectin will be shorter or be smaller, so it will influence the, I say the tubidity or some. In a good or a bad way? I cannot really tell, because if everything, every pectin degraded, it will provide the, I say the dietary benefit as a probiotic benefit but it will influence the quality or the physical quality so we cannot really tell it's a good way or a bad way. Yeah, so still a lot of additional research to be done. Another question is that for the pectin, you now did your study on the structural characterization of the hot water extractable and the chelator extractable pectin fraction, but there is also still the pectin fraction which is bound very tightly, that can only be extracted by alkali. You did not consider that fraction. Can you motivate this choice, please? Yes, at first, actually we did extract with alkali but you know that alkali will satisfy the pectin structure and there will be no mechanism over the pectin backbone so use our method, there is all the blocks so we didn't look that way but let's say from our lab mate and they investigate this alkali, we extracted the pectin with some other character juice of some others and they have some results but I can't, it's not in my thesis, so. Yeah, so yeah, it's difficult indeed to isolate them in a way that they are not structurally changed but in your opinion, what is their contribution towards the juice quality characteristics that you have determined? Will they still play a role there or not and how large was this fraction? Because that you could have determined how large is this solidly bound pectin fraction that you did not characterize compared to the whole pectin fraction? Yeah, certainly they will affect a lot because they are the remaining, let's say, the macromolecules there and they will, because they are in a high molecular weight, they will settle over time so it will influence the total quality of the product and yeah, in the future, we cannot really remove these molecules so in the future but I said, let's say we cannot really find their role in stabilizer or, as a stabilizer or a sickening agent so, yeah, so we cannot ignore them but we will find a way to, how to solve them or maybe do more filtration or something like that to remove this interference. Yeah, okay, thank you. Thank you, Professor von Louis. The opposition will now be continued by Dr.

Foltz who is Global Head of R&D at the Dole Group in Darmstadt in Germany, the floor is yours. Thank you for the introduction, the respected candidate, also thank you for PhD thesis, significant amount of work you conducted and also a lot of contribution to science, thank you for this. In my questions, I would like to come more to the industrial valorization of the results and first and foremost, I come also back to the question Professor Fokliani was phrasing in the beginning and I would like to emphasis or come back to, why have you been selecting these 13 different type of apples and what do you think today is taken as a parameter in the industry to use apple for apple processing to make juice, purees and what you describe next to the cultivars. Okay, I assume the opponent, thank you for your next word and the questions. Yes, we are selecting these 13 cultivars based on like some of the cultivars are widely planted in the like say the Fuji or the Pink Lady or the Golden Delicious, they are widely planted cultivars and then we also selected some cultivars that are newly bred breeding in China so we add this kind of a mixture of the combination. So, but yeah, as far as I know, in the industry, they use Maori in Guarner's and Golden Delicious, I think. In the industry, we don't use cultivars so we use different parameters who primarily drive on yield in the choosing process. So the fruits, what you describe are primarily table fruits. So desert fruits, you consume and buy in the supermarket but in a factory, yeah, we buy bulk fruit and in most cases, it's not the cultivar what describes it but different parameters. So it's breaks, for example, what drives then also the consumption of those apples. But let's continue and go to different aspects in your work and also relates to practical questions and in your work, on one hand, you look at two different cultivars and then you say, I'm going to standardize for the ripeness staging, yeah. It's a little bit similar like to, let's say you have a chronological agent, you have a physiological agent. So what could be parameters to

standardize for ripeness in an apple? Yes, in our research, we can only obey because we are not the expert in planting apples. So our experience from farmers experience, they told them which are in the, let's say the similar maturity so we can obey their suggestion and collect this different cultivars. And yes, for the maturity, we should, let's say, use more centrifugal waste, let's say the... Can you be a bit more specific? So for example, if you look at your work, could certain, let's say, activities in an apple describe the physiological age of that apple? Yes, I checked the literature, then maybe they can use some to test the content of the starch or the content of other, let's say the textures to... Are there others in relation to enzyme activity, for example? Maybe for the, because I didn't really look here, but yes, as far as I know, that we can use some enzyme test and to test the enzyme of the pectinase and the PPCO also, maybe. So if you look at the activities and you are alluding to pectinases, and if you take a specific example, PME, for example, if you look at the cause of the ripeness of the apple, what happens to PME activity in cause of the lifetime, is it all equal or does this differ? Yeah, this certainly will change it because let's say that it's a dynamic processing or post-harvest regulations and the PME will, as far as it will increase and then go down because there is no mass research left. So maybe PME is an option for that maturity. Yeah, and that's also what happens in practice in the industry. So we look at, in apple processing, different enzyme activities, but also pectin structure, not as sophisticated as you do in your working, but it's very important. And also starch, what's the starch accreditation process, because also we use enzymes in the process as such, so very important aspect to look at. And I would doubt a bit whether the variability you see across cultivars is more relevant than the variety you see over the season in the apple, so to adapt that in processability. Also, when you look at the measures you take in your work and the analytical equipment, what you're using, this is very elaborated, this is very sophisticated, it's of non-practical use in a factory. Could you think of systems which could be applied and be taken also for in-line processing to take some of the elements you have been doing in your work to apply it also today in industry processing of apples? Yes, because we made our juicing live, we cannot really, because we have only a few samples, we cannot really go to the industries or the factories to process them. And we already try to mimic the real processing ways already, but so the differences between our live work, live scale and the factory scale is the processing, the preparation, I think in industry they are using more, the press, but we are using only the simple juicer, so. Yeah, again, a bit more specific, so for example, if you look in your chapter six in most of your work, you use HPAC, PAD, but you also refer to using NRI and FDR, so it's back to corpus bands, and which of those would correlate most strongly with your degree of blockiness, Markus? Yes, our research is based on the results of the HPAC and the helix. This cannot be achieved in the industry, I say it can only be achieved in a lab, and it's, yeah, it's difficult to, in the industry they can not really use this method because it's too accurate and needs some practical work, so it's what we want to do, that we provide general information that which culture has which kind of information to the factory or to the industry, and they can use our information, but not applying the method. But NRI, would it be a possibility to apply in a factory? I should say, yeah, it's, to be honest, it's easier to direct, to test the physical parameters of the products, not to the fundamental material, the molecules there, so I think it's, it could be better, it's quite hard. I think it can, and in part it's done, but requires a lot of testing, a lot of big data set, and it takes companies years to acquire those, but then it can help, yeah, so it's done. So I would like to come to another part of your work which also relates to using quite heavily the Xbox model for calcium-packed cross-linking. If you look also at RG2, not at RG1, but at RG2 dimerization, could be there also another theory, also in combination what you do experimentally in your work using EDTA as a shellator, which could explain also different model by which this shellating could happen, also in relation to RG2? To be honest, we didn't look into RG2 or RG1, we only, let's say, as I mentioned previously, that we firstly focus on the. By doing this, did you not omit an important part of that shellating aspect, like shellating

by boron through EDTA? Yeah, it's important that our major goal is to find out the difference, major in the distribution patterns, so. And then again, thank you for this, again, I would come back to a part where the thesis is very heavy on, let's say, also yield optimization and cultivar selection, and you also elude in your work to the Pink Lady, which is a nice digital apple, which I also prefer, and you elude, it could be a preferred choice, also for a cloudy juice, and based on your work, could you think of and come up with a sort of processability index where such an apple then should go into cloudy juice production, and where not? And what would be parameters again in that processability index? Yeah, we found some certain cultures that are suitable for the processing, because if you want more cloudy juice, the stability is important, so we try to use some cultures with higher, in our words, it's blocks, with more blocks, and if some people would like the separation, because it's nature appearance, they will use less blocks, I suppose. But I cannot give the name, the apple name here. And then I would like to continue and go away from the apple, and go into a different direction, so extending the Pectin Metabolite framework you described in chapter five and chapter six. If you link that to Pectin metallation and organic acids, can you also suggest a regulatory link between? I'm afraid that we'll have to stop it here, but I'd like to thank you very much, Dr. Foltz, for your opposition. I now adjourn the meeting, the examining committee, we will draw for consultation. Please be seated. I hereby reopen this meeting. The Academic Board of Wageningen University, represented by the Deputy Director of Magnificus, and six committee members appointed by the Academic Board, having noted the content of a thesis entitled Characterization of Apple Pectin Structure in Relation to Apple Fruit Processibility with Propositions, having heard the defense of that thesis, has decided to confer the degree of doctor on Daju Liu, born in Yidin, China, on October 31, 1990, and to grant to him all rights and privileges ensuing from that doctorate by law and customs. The Academic Board assumes that you accept your duty as a scientist to execute your future research ethically and with due diligence, according to the Netherlands Code of Conduct for Research Integrity. I now invite the promoter, Dr.

Foltz, to present the new doctor with the degree. You've heard the decision of the Academic Board of Wageningen University to confer on you, Daju Liu, the degree of doctor. It's now my honor to present you with a degree signed by the Deputy Director of Magnificus, the promoter and co-promoter, and seal to his great seal of Wageningen University. I first invite you to sign the degree as well. This is a signature you declare to act according to the Netherlands Code of Conduct for Research and Ticketee and in the future. Allow me, Mr.

Director, to offer my congratulations and to add a person with this. Dear Dr.

Liu, dear Darcy, it's my pleasure to be the first to congratulate you also on behalf of the co-promoter, Professor B, and your supervisor, Dr. Xihan Liu, the you most from the Chinese Academy of Agricultural Sciences in Beijing. This obtaining the degree of doctor, a great achievement after so many years of hard work. I would like to extend our congratulations to your wife, Jenny, Liu, also a Liu, present here in the audience, to your friends, also present here today, and to your parents and for the family and friends watching this ceremony online. Darcy, your research was the first PhD research at Food Chemistry within the WURF CAS, Sandwich PZ Program, and in 2070, you started your PhD on apple cell wall structures. A wide range of apple varieties were harvested to process in China into apple juice and pomace. You were involved in the processing, but more importantly, you focus on, to understand how cell wall pattern affected the physical properties. You zoomed in on the molecular structure of packed polymeric, polyclacto-romic acid mainly, in particular on the distribution of petile acids. In all the different packed populations, I will not say thousands, but quite a few, isolated from all the different varieties tested from two different harvest years. You found huge differences between material acidification distributions present in apple, present in apple as in solo calcium packed in gel. Using many statistical techniques, you

demonstrated the relationships between packed in structure, physical properties like particle size, particle charge, and viscosity, as you have heard. You teach us three scientific papers or they publish in the high impact journals in the field, and one scientific chapter laying the first, very first basis to use apple metabolomics to predict apple packed in structure and physical properties of the product. You will also co-author of several other papers from Professor Bee's lab in Beijing, in many cases also co-authored by your wife, Tianning. The thesis can be considered as a valuable piece of research enabling us to further study packing structure and functionality physical properties. And now some more personal words. You really deserve the doctor title, not only because of all the nice research we discussed today, but you were certainly determined and patinacious to finalize your PhD after all these years of delay, other duties, and other priorities. Your adventure in bargaining is started by a request from Professor Bee from the Institute of Food Science and Technology to start collaboration between the two groups within the framework of the Workhouse Center with the program. You were proposed by Professor Bee as a good candidate, especially since you already received your training and awareness of the do's and don'ts while doing a PhD in bargaining in Beijing. You started your research in 2017 China and arrived in bargaining in 2018 June for a stay of six months. Back in China, you'd process all the different apple varieties and collected too many packing samples to be collected as bargaining during your second stay from September to March, 2020. Just like COVID started, you arrived in China and you experienced a really heavy lockdown as a long period where you could, but could hardly work on your thesis. And where even forces stay in a separate place. In August 21st, you and I have back in bargaining for the second time, and you can actualize all your patents, and thanks to a weird extension, you extended your stay till 2023, December. In your research, you really observed, you really observed, confirmed and illustrated the old provoking statement of our first food chemistry professor Walter Pilnick that no patent molecule is alike. Partly due to this high complexity of patent structure, we had quite some difficulties to put our first paper published. It really frustrated you. We succeeded in 2023 with as consequence that you really had to do a lot of writing after project ends and after having arrived in China. And this was quite hard for you, especially since you also accepted the position as scientist in the starting up company. Last year, you committed and dedicated yourself to writing only and finalize your thesis. Also, in your personal life, you sometimes failed a bit in being as efficient as possible. You were a part of Zhenning. We're extremely lucky. There's a golden chance that also Zhenning could do a sandwich piece of Zhenning and a group of professor folk piano. However, the two of you succeeded for the first two years to have the Zhenning a visit of the one coincided with a visit in Beijing for the other. So it's not that efficient. But still, together you collaborated a lot with several joint papers, and you even found time to meet in China to get married in a traditional way. During your Zhenning periods, you integrated nicely with all your colleague PC students, and you joined two PC trips. During one of the PC trips abroad, we visited Austria, the Italian, Italy, and in France, we were extremely happy to have you in the team. Since you did your part in BSE, but your master's at the University of Camarino in Italy. So you would play a role as local Italian, we hope. However, partly due to your modesty, you did not really interact much with Italian folk, forcing us to find another guide to show us the way. You certainly joined all social events of food chemistry, all parties, and every week's practice to go to the food of that day of that week. In contrast to your, now a then slightly hidden presence at food chemistry, you were known, I believe, to every Chinese MSE and PC student in marketing, being there or having been there. So you know them all. When I was looking for a correct person or a special contact from China, you know where to find and where to search. Nancy, we wish you a wonderful future, first celebrating a bit your degree and back in China, finding a job in the same city where Zhenning has been offered a permanent position. You mentioned that job finding might be a bit challenging because of the city being relatively small. Well, China, only eight million people. All the best and have a wonderful day today. And here it is, I give

the word back to the director. Thank you, Professor Scholz. Dear Dr.

Liu, it is also my pleasure and honor to be able to congratulate you with your just obtained title and also I would like to include your wife in this congratulation. I'm sure your wife, but also your family in China is very proud of your achievement today. And so are we as university because with your work you have been able to contribute to our motto to explore the potential of nature for quality of life. In this case, to look at the properties of apples and infections in these apples. And as a food scientist myself, I was interested to hear your findings and also the discussion today. I think that what you have been done is really a basis. There were also questions about practical applicability and you mentioned that this is the basis on which we need to further do research on this to also apply it in the industry, but I'm sure that that will happen at some point. You also told me that you are interested in finding a job within industry and I suppose then would be the food industry, maybe even the apple industry. And I wish you lots of success to find a suitable job for you in the future. I'd like to thank the committee for their time and efforts in evaluating the thesis, but also to be present here today. Professor Quimber coming from Portugal, Professor Van Lee from Belgium and Dr.

Foltz from Germany. And of course, also our own professor, Fokudiano from Wageningen. It is much appreciated that you took the time and that we could use your expertise to evaluate the PhD work done here. And finally, dear audience, I'd like to thank you for witnessing the graduation of Dr. Liu. And now it is time to celebrate and so I close this ceremony.