Wageningen, August 10, 2010

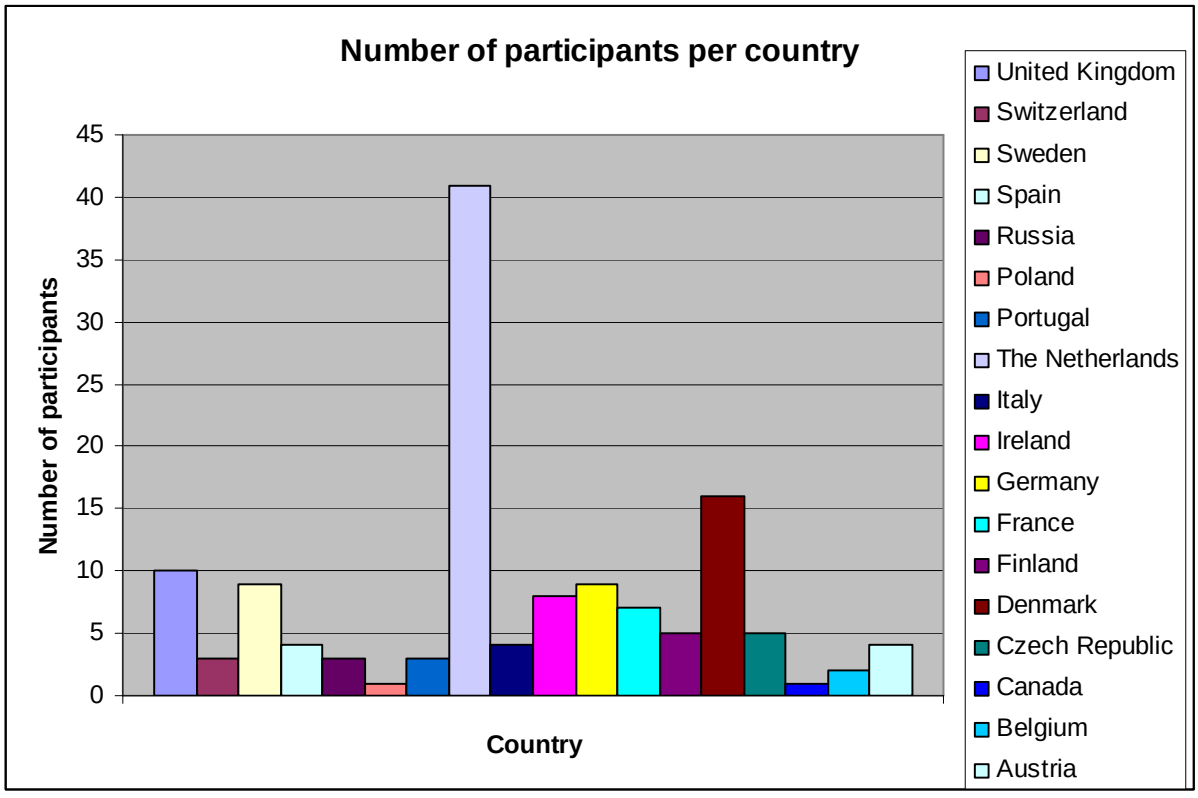
Scientific Report (*Final version*)

Reference:	**3038**
Science Meeting:	School
Title of Science Meeting:	Summer Course Glycosciences
Location:	Conference Centre Hof van Wageningen, Wageningen, NL
Date of Science Meeting:	17 – 20 May 2010
Convener Name:	Professor Alphons Gerard Jozef Voragen, Wageningen, NL

Local Organizers:	Chantal Doeswijk, Graduate School VLAG Dr. Henk Schols, Laboratory of Food Chemistry, Wageningen University
Address:	Bomenweg 2 6703 HD Wageningen The Netherlands Chantal.Doeswijk@wur.nl

1. Summary

The Summer Course Glycosciences is a bi-annual event that was organized for the 11th time this year by close collaboration of Wageningen University, Groningen University, CERMAV-CNRS and Grenoble Universités / ESRF.
Distinctive for the Summer Course Glycosciences is that general introductions in the field of polysaccharides and glycoproteins are combined with in depth parallel sessions. This gives participants the possibility to focus on specific interests without loosing a broad education.
Another feature of the Summer Course is that most lecturers will be present during the course for further communication with participants. In addition, within the programme 20 – 25% of time was scheduled for discussions.
This edition the participants were exposed to 15 plenary sessions and eight parallel sessions, alltogether 31 lectures given by 21 senior scientists from six European countries.
In general the Summer Course attracts about 100 particpants. However this year we exceeded the number of available places (115) three weeks before the registration deadline. Luckily we were able to re-arrange some of the logistics so we could welcome another 20 participants from the waiting list resulting in a final count of 135 participants from 18 different countries!



All particpants and lecturers were given access to a special Glycosciences website were they could find course information and download background literature prior to the course. In addition nearly all presentations were made available as PDF-files within two weeks after the course. The website will be open to all participants for at least one year. A course binder containing hardcopies of the presentations, a Book of Abstracts and a printed Book Chapter were handed out to all participants and lecturers during registration.

2. Scientific content and discussion

Introduction

The 2010 Summer Course Glycoscience (11th European Training Course on Carbohydrates), held from May 17 to May 20, 2010, in Wageningen, The Netherlands, comprised a well-balanced educational programme for the 135 participants (master students, PhD students, post-docs, lecturers, industrial scientists) from all over Europe. The topics were grouped in 6 topical research areas:

- Carbohydrate Synthesis and Biosynthesis
- Carbohydrate Analysis
- Carbohydrate Degrading Enzymes
- Polysaccharides and Functional Properties
- Medical and Health Applications
- Valorization of Carbohydrate-rich Biomass

Carbohydrate Organic Synthesis covered an Introduction to Carbohydrate Synthesis and an Advanced lecture on Chemical Synthesis of Oligosaccharides by Prof. Dr. S. Oscarson. He gave a general background and focused on three areas of major importance: protecting group manipulations, stereoselective glycosylation reactions and synthetic strategies.
Prof. Dr. S. Kelm gave a basic introduction in the ***Biosynthesis*** of glycoprotein N- and O-linked carbohydrate chains, as well as on the importance of carbohydrate-recognizing proteins worked out with examples from the selectin field, whereas Prof. Dr. H. Höfte presented an overview of our current understanding of the ***Biosynthesis*** of cell wall polysaccharides using genomics of the model species *Arabidopsis thaliana* for the identification of key players in cell wall synthesis.
Prof. Dr. Monica Palcic gave an overview of Enzymatic Synthetic methods that can be employed for the preparation of oligosaccharides. Strategies for the synthesis of a variety of natural oligosaccharides and analogues of natural structures were covered using e.g. glycosyltransferases, glycosidases, engineered glycosynthases and whole cell metabolic engineering.

Carbohydrate Analysis was introduced by Prof. Dr. J. P. Kamerling who gave a systematic approach for the elucidation of the primary structure of carbohydrates. Chemical and enzymatic approaches for both polysaccharides and glycoprotein glycans were discussed.
Prof. Dr. T. Peters provided basic knowledge about carbohydrate NMR. This included the fundamental properties of carbohydrate ^{1}H and ^{13}C NMR spectra, and how to derive conclusions about e.g. anomeric configurations or types of glycosidic linkages from such data. Also concepts that aim at sequence and conformational analyses were introduced e.g. the structural reporter group concept. Important novel NMR technological developments and their impact on the analysis of carbohydrates were explained. Such developments are the dramatic improvement in sensitivity and the availability of specialized pulse sequences that deconvolute crowded carbohydrate NMR spectra.
Prof. Dr. R Geyer illustrated the increasing importance of mass spectrometry in carbohydrate analysis due to recent developments and instrumental improvements enabling hyphenated techniques, such as nano-liquid chromatography (LC)-mass spectrometry (MS). After addressing the general difficulties mass spectrometry was confronted with in carbohydrate structure analysis, three selected topics were worked out: (1) A brief survey of strategies suitable for a glycomics type of analysis of tissues and organs or glycoproteins derived thereof, (2) a short introduction into key mass spectrometric techniques and (3) applications of the outlined techniques for the structural analysis of N- and O-glycans. Different mass spectrometric approaches were discussed demonstrating the great power, but also the inherent limitations of these techniques.
Prof. Dr. O. Blixt gave an overview of the use of glycan microarrays in the analysis of carbohydrate-protein interactions. After an introduction of the different chemical approaches of preparing suitable glycan microarrays, mentioning advantages and disadvantages of the various protocols used, he focused on their high-through applications in glycobiology and glycomedicine. Several examples were

presented of the usefulness of glycan micorarrays as a first step in tracing and understanding biological interactions in which carbohydrates play a major role.
The three-dimensional aspects of complex carbohydrates, alone or in their interaction with proteins, were highlighted by Prof. Dr. T. Peters, Prof. Dr. S. Perez, and Dr. M. Frank. Prof. Peters covered ligand-based NMR experiments for the analysis of carbohydrate-protein interactions. Transfer NOE experiments and saturation transfer difference (STD) NMR experiments were explained in detail and applied to selected examples. It was shown that this methodology is very well suited for the application in biological systems since the targets can be e.g. whole cells or viruses. Prof. Perez explained the basics of molecular modeling using molecular mechanics and molecular dynamics approaches, whereas Dr. Frank gave a series of applications of molecular modeling, thereby demonstrating the importance of such approaches in understanding interaction phenomena in the glycofield.
Finally, Dr. M. Frank discussed the need and potential of databases and software for Glycosciences. Glycobioinformatics is still in an explorative stage, but his lecture gave many openings for the participants how to incorporate databases in their research.

Carbohydrate degrading enzymes was introduced by Prof. Dr. M.O. Couthinho who discussed criteria and systematic of classification and annotation of carbohydrate degrading enzymes. In an additional lecture he gave a state of the art overview of carbohydrate binding modules and enzyme modularity. The structural and functional analysis of carbohydrate acting enzymes was discussed by Prof. Dr. B.W. Dijkstra. X-ray crystallography, the construction of designer mutants of the enzyme and the availability of crystallized enzymes with embedded substrate were illustrated to be of key importance in this analysis.
Dr. G. Beldman covered the plant cell wall polysaccharides (i.e. cellulose, pectin and hemicelluloses) degrading enzymes. Several aspects of plant polysaccharide degrading enzymes were presented, in particular their mode of action towards the complex cell wall polysaccharides in relation to their application in food and bio-based technologies.
Enzymes active on starch were discussed by Prof. Dr. M.J.E.C. van der Maarel. After a general introduction on the chemistry, physical structure and changes therein upon heating in aqueous media, industrial processing of starch to maltodextrins, maltose and glucose syrups using starch degrading enzymes was discussed. Hydrolyzing enzymes that are able to modify starches by transglycosylation reactions were also covered, e.g. cyclodextrin glucanotransferase and amylomaltase. In a second contribution Prof. van der Maarel addressed the enzymatic conversion of renewable, carbohydrate-rich raw materials into valuable products. Examples are the conversion of sucrose to glucans with different glycosidic linkage types by glucansucrases from various lactic acid bacteria, the use of α-amylases with a starch binding domain for the conversion of starch to fermentable sugars at lower temperatures and the use of complex mixtures of cellulases from fungi for producing fermentable sugars from cellulose and hemicelluloses.

Polysaccharides and their functional properties dealt with plant cell wall polysaccharides, bacterial polysaccharides, the storage polysaccharides starch and cellulose. Dr. H.A. Schols gave a short description of the most dominant polysaccharides as present in different plants (monocotylic versus dicotylic plants; primary versus secondary cell walls), the cross-links within the cell wall between different (classes of) polysaccharides, their isolation and characterization and detailed information on the structural elements of pectin and the wide variety of variations possible within these structural elements depending on origin and localization within the plant. In addition to the carbohydrate analysis lectures, mentioned already above, examples were given of how to approach the elucidation of the chemical structure of such highly complex and highly variable plant polysaccharides by making use of chromatography and pure and well-defined carbohydrases in combination with identification of oligosaccharides released using state-of-the-art analytical techniques like e.g. mass spectrometry. The polysaccharide composition and chemical structure and architecture of cell wall polysaccharides were

related to the functional properties of (isolated) cell wall polysaccharides and the quality of foods derived from plant produce.
Prof. Dr. O Holst in his lecture gave an overview of the broad variety of polysaccharides found in bacteria, in particular with a focus on structures, functions, and biosynthesis of polysaccharides from Gram-negative and -positive bacteria, and from mycobacteria, e.g. lipopolysaccharides, capsular polysaccharides, lipoteichoic acids, arabinogalactan, and others. Together with this, the general architectures of the Gram-negative, -positive and mycobacterial cell walls were discussed.
Finally, Prof. Dr. S. Perez reviewed the state of the art of the structure and morphology of cellulose. After a general discussion on plant cell walls, he focused on different types of cellulose, their biosynthesis, their microfibrillar state, and their analysis using different technologies, such as X-ray diffraction, electron diffraction, and neutron diffraction. One of his take-home messages was: In all interactions of cellulose with external reagents, its morphology plays a central role to determine or even predict its reactivity. Dr. P. Buwalda explained how the complex architecture of starch granules correlated with starch functionality. The crystallinity of the amylopectin part of starch is influenced not only by natural variations like phosphate and lipid levels, but can be changed upon chemical modification of the starches. The role of cross linking and stabilisation of starches to improve their functionality in food and non-food applications was discussed.

Biomedical and health applications Dr.C.H. Hokke lectured about the use of the recently discovered carbohydrate-based biomarkers (glycan biomarkers) and the development of associated technologies. These are based on changes in glycan profiles of glycoconjugates in body fluids (e.g. serum glycoproteins) and may be indicative of biological conditions or changes in cells, organs or organisms e.g.inflammation, liver cirrhosis, different malignancies, or ageing. Specific examples are the changes to the overall serum glycome induced by various types of cancer, as well as the decreasing galactosylation of immunoglobulin G Fc glycans during ageing. Direct or indirect detection of pathogen-derived glycans secreted into the circulation and ultimately in the urine of infected hosts can also serve as infection marker.
The lecture also addressed glycan biomarker discovery programs which usually require relatively high throughput methods to be able to deal with the large sample sets necessary for reliable statistical evaluation. This was illustrated by several applications, including the overall serum glycome profiling using LC with fluorescence detection, the detection of parasite-derived glycans by a combination of affinity chromatography and MS, and the specific analysis of IgG Fc glycopeptides in relation to a number of immune diseases as well as aging.
Professor Dr. J. Garssen discussed the benefits of non-digestible oligosaccharides on the immune system starting with an introduction to the immune system. He distinguished a non-specific first line defense, a non-specific innate immunity and finally a specific adaptive immunity. Their development, function, action and interaction as well as the consequences of its failure were described. Results of recent studies of prebiotics on the immune system were then discussed. Their indirect (through the composition of the gut flora) as well as direct effect on the immune system were demonstrated with results of studies. Prebiotic mixtures of scGOS/lcFOS were found to affect both DC/T cell interactions, macrophages as well as TLR9 stimulation in a direct way without any bacteria or bacterial products available. scGOS/lcFOS were also shown to lead to highly significant effects on infections as well as allergies.

Valorization of carbohydrate-rich biomass Dr. M. J. O'Donohue lectures dealt with Carbohydrate bio refineries - concepts, technologies and process configurations and Industrial bio-transformation of sugars into value-added fuels and chemicals. In his first lecture the notion of biorefining was explained and some basic facts and figures were supplied to help the audience to get a basic understanding of the issues at stake. Starting with 1st generation biorefining, some basic process concepts were introduced followed by a discussion of 2nd generation biorefining. The hurdles and drawbacks of the different technologies were underlined and a view of some future prospects in this exciting area was presented.

The second lecture provided a general overview of Industrial Biotechnology with some basic figures which gave the audience a grasp of the current status and future potential of this technology. Further, modern technologies that underpin the creation or improvement of biocatalysts were outlined and several examples of existing biotechnologically-driven processes were discussed. The lecture was concluded by featuring future frontiers, especially concerning the prospect of harnessing microbial consortia. It is expected that Industrial Biotechnology will be a key driving force in a bio-based economy, which will use carbohydrates as the principal feedstock for fuels and chemicals.

Poster Sessions During the meeting 2 sessions of about 2 hours each were held where the participants showed posters of their actual research work. These were very successful sessions with lively discussions between participants and with the lecturers. Participants were challenged to select the best posters which they did with a broad consensus. These sessions were highly appreciated by all participants.

3. Assessment and impact:

All particpants were asked to fill in an evaluation form and give an overall rate of the course varying from excellent to poor. The course was rated as excellent by 37% of the respondents, as good by 58% of the respondents and as moderate by 5% of the respondents.
97% of the respondents were very staisfied with the amount of information that was provided by us (background literature and handouts in course binder). 80% of the respondents indicated that the course met up to their expectations or even more. 16 % indicated that they had expected some more focus on synthesis or biomedical topics but were furthermore satisfied. 88% were very satisfied about the framework of the course. The other 12% would have appreciated some more breaks and free time. Finally, 97% of the respondents found the special Glycosciences webpage very usefull.
It also became evident from the evaluations forms that the course is indeed advanced and at graduate level and interesting for those working in research as well as industry. The participants indicated that they really enjoyed the interaction between the participants and lecturers and some even recommended smaller parallel session with more interaction.

In view of the high number of attendants to the course and the results of the post-course evaluation it can be concluded that this course clearly meets the need of young glyco-scientists for on one hand a broader background in glyco-sciences and, on the other hand the need for more specific knowledge in specific areas of glyco-sciences.
The course also contributes to formation of networks and collaborations on several levels:

- Between young European scholars and renowned, senior scientists teaching the courses.
- Between young, starting European glyco-scientists
- Between European science institutions on Glyco-sciences

From the evaluation it could also be concluded that the existing course needs continuous updating. The students expressed for future courses more attention for bio-medical aspects. There is also a growing interest for a “glyco-based” economy.

4. Final programme:

Monday May 17, 2010

09.30 – 10.20	Registration, Poster mounting	
10.20 – 10.30	Opening Summer course Glycosciences	Prof. Dr. L. Dijkhuizen
10.30 – 11.30	Plenary session 1: Introduction to plant cell wall synthesis	Dr. H. Höfte
11.30 – 12.30	Plenary session 2: Introduction to glycoprotein biochemistry	Prof. S. Kelm
12.30 – 13.30	Lunch	
13.30 – 14.30	Plenary session 3: Introduction to carbohydrate synthesis	Prof. S. Oscarson
14.30 – 15.30	Plenary session 4: Molecular modeling of carbohydrates,	Prof. S. Perez
Coffee/tea break		
15.45 – 16.45	Parallel session I A: Advanced oligosaccharide synthesis	Prof. S. Oscarson
15.45 – 16.45	Parallel session I B: Structural aspects and properties of cellulose	Prof. S Perez
16.45 – 17.45	Plenary session 5: Introduction to bacterial polysaccharides	Prof. O. Holst
Coffee/tea break		
18.30 – 20.00	Dinner	
20.00 – 21.30	POSTERS and drinks	

Tuesday May 18, 2010

08.45 – 09.45	Plenary session 6: Primary structural analysis of carbohydrates	Prof. J.P. Kamerling
09.45 – 10.45	Plenary session 7: Introduction to plant polysaccharides	Dr. H.A. Schols
Coffee/tea break		
11.15 – 12.15	Plenary session 8: Carbohydrate degrading enzymes: classification and annotation	Prof. P.M. Coutinho
12.15 – 13.15	Lunch	
13.15 – 14.15	Plenary session 9 : Carbohydrate –recognizing proteins	Prof. S. Kelm
14.15 – 15.15	Plenary session *:	Dr. M. Frank
Coffee/tea break		
15.45 – 16.45	Plenary session 10: NMR methodologies and strategies in glycoscience	Prof. T. Peters
16.45 – 18.30	Free time to explore Wageningen	
18:30-20:00	Dinner	
20:00-21:30	POSTERS and drinks	

Wednesday May19, 2010

08.45 – 09.45	Plenary session 11: Mass spectrometry in glycoscience	Prof. R. Geyer
09.45 – 10.45	Plenary session 12:Carbohydrate bio refineries - concepts, technologies and process configurations	Dr. M. J. O'Donohue
Coffee/tea break		
11.15 – 12.15	Parallel session III A: Carbohydrate-based biomarkers	Dr. C.H. Hokke
	Parallel session III B: Structural and functional analysis of carbohydrate acting enzymes	Prof. B.W. Dijkstra
12.15 – 13.15	Lunch	
13.15 – 14.15	Parallel session IV A: NMR spectroscopy of carbohydrate-protein interactions	Prof. T. Peters
	Parallel session IV B: Carbohydrate Binding Modules and enzyme modularity	Prof. P.M. Coutinho
14.15 – 15.15	Parallel session V A: Enzymatic carbohydrate synthesis	Prof. M.M. Palcic
	Parallel session V B: Bio-Chemical properties of starch	Dr. P. Buwalda
Coffee/tea break		
15.30 – 16.30	Parallel session VI A:Biomedical aspects of (poly)-saccharides	Prof. O. Holst
	Parallel session VI B: Starch active enzymes and their application	

		Prof. M.J.E.C. van der Maarel
16.30 – 17.30	Plenary session 13:Glycoinformatics - databases and software tools for glycosciences	Dr. M. Frank
18.30 – 23.00	COURSE DINNER with Poster Prize awarding	

Thursday May 20, 2010

08.45 – 09.45	Plenary session 14: Glycan microarrays	Dr. O. Blixt
09.45 – 10.45	Parallel session VII A: Plant cell wall polysaccharide degrading enzymes in food and non-food applications	Dr. G Beldman
	Parallel session VII B: Carbohydrate bioconversion	Prof. M.J.E.C. van der Maarel
Coffee/tea break		
11.15 – 12.15	Parallel session VIII A: Glycosyl transferases and blood groups	Prof. M.M. Palcic
	Parallel session VIII B: Industrial biotransformation of sugars into value-added fuels and chemicals.	Dr. M. J. O'Donohue
12.15 – 13.15	Plenary session 15: Non-digestible carbohydrates: an immunity nutrition breakthroug	Prof. Dr J. Garssen
13.15 – 13.30	**Closing session**	
13.30 – 14.30	Farewell Lunch	

*The two sessions below were cancelled because Prof. Williams was not able to attend the course due to the vulcano eruption. One of his sessions was replaced by a plenary lecture of Dr. Martin on the applications of molecular modelling.

Plenary session 5:	Physico-chemical properties of polysaccharides	Prof. P.A. Williams
Parallel session II B:	Interactions in mixed polymer systems	Prof. P.A. Williams