Project BioFoamBark
VTT Results summary

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BioFoamBark project and VTT aims

Full title	Bark valorization into insulating foams and bioenergy
Call	Joint Call of WoodWisdom-Net and ERA-NET Bioenergy: "Sustainable forest management and optimised use of lignocellulosic resources" (2011)
Duration	1/2012-12/2014
International consortium	University of Freiburg (coordinator, Germany), University of Ljubljana (Slovenia), Université de Lorraine (France), University of Santiago de Compostela (Spain), Fraunhofer Institute of Solar Energy Systems ISE (Germany), Nova-Institut GmbH (Germany), Ledoga Srl (Italy)
Total budget	
VTT budget	240 k€
VTT funding	Tekes 60%, VTT 25%, Savanaho Oy 8%, Finnish Wood Research Oy 4%, Chemigate Oy 3%
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- **VTT's specific aims** determined in the project plan:
  - to optimize the hot water extraction procedure for each relevant bark tannin.
  - to develop economic enzymatic purification method for hot water extracted tannin in laboratory and pilot scale.
  - to produce crude and purified tannin in pilot scale and to supply it to other partners.

Content of the summary

- Raw materials and their compositions
- Effect of storage on spruce bark composition
- Optimization of hot water extraction in bench scale
- Pilot-scale extraction of spruce bark
- Purification of crude tannin
    - Hydrolysis
    - Ultrafiltration
    - Solvent purification
- Composition of crude and purified tannins
- Conclusions
- Dissemination of results

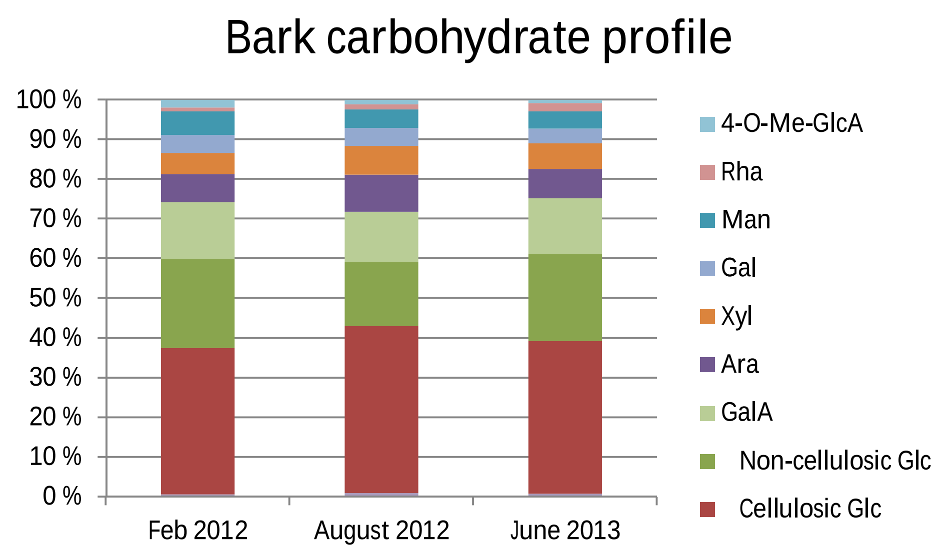
Raw material

- Dry-debarked spruce bark was obtained from Metsä Wood Kertopuu plant in Lohja (Finnish Wood Research Oy owner company).
- The bark contained 10-21% wood. Wood impurities were present in pilot-scale extractions but they were removed manually before bench-scale extraction experiments.
- Three batches were acquired:
  - February 2012 → bench-scale extraction
  - August 2012 → 1st pilot-scale extraction
  - June 2013 → 2nd pilot scale extraction

Spruce bark composition

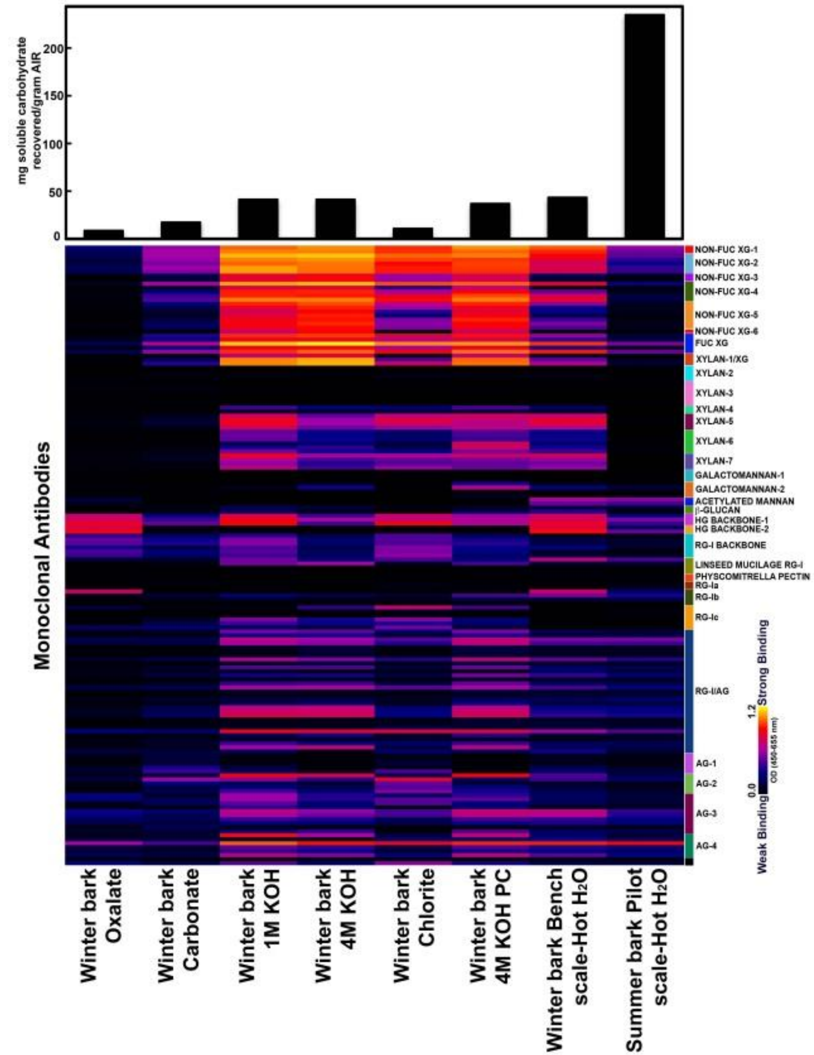
- Spruce bark contained 48-51% carbohydrates, 33-37% lignin, 2-3% lipophilic extractives, 3% ash and circa 11% tannins.
- Bark carbohydrates were mostly composed of cellulose, non-cellulosic glucose, pectin and minor amounts of other hemicelluloses.
- Collection time did not appear to have a major effect on the overall composition of spruce bark.

Collection time	February 2012	August 2012	June 2013
Carbohydrates	51.3	47.5	49.9
Acid insoluble lignin	31.1	33.5	30.7
Acid soluble lignin	2.7	3.3	2.3
Lipophilic extractives	2.9	2.4	2.6
Ash	3.1	3.1	3.3
Tannin	n.a.	10.7	11.7
SUM	91.1	100.6	100.6



Spruce bark composition

- The glycome profile of spruce bark was investigated in collaboration with Sivakumar Pattathil from Complex Carbohydrate Research Centre, University of Georgia, USA.
- A set of nearly 200 hemicellulose antibodies were screened against chemical and water extracts to reveal the type and binding strength of polysaccharides in spruce bark.
- The study showed the presence of xyloglucan, pectic polysaccharides and arabinogalactan in bark.



Effect of storage on spruce bark composition

- Bark obtained for the first pilot-scale extraction experiment was stored outside for 10 months over the winter.
- The storage:
  - Significantly decreased the neutral carbohydrate content
  - Significantly increased the acid insoluble lignin content
  - Decreased acid soluble lignin content
  - Decreased tannin content
- Possible causes:
  - Microbial activity consumed carbohydrates
  - Aging caused condensation/polymerization of tannins to acid insoluble material
  - Water soluble tannins and sugars were washed away in wet conditions

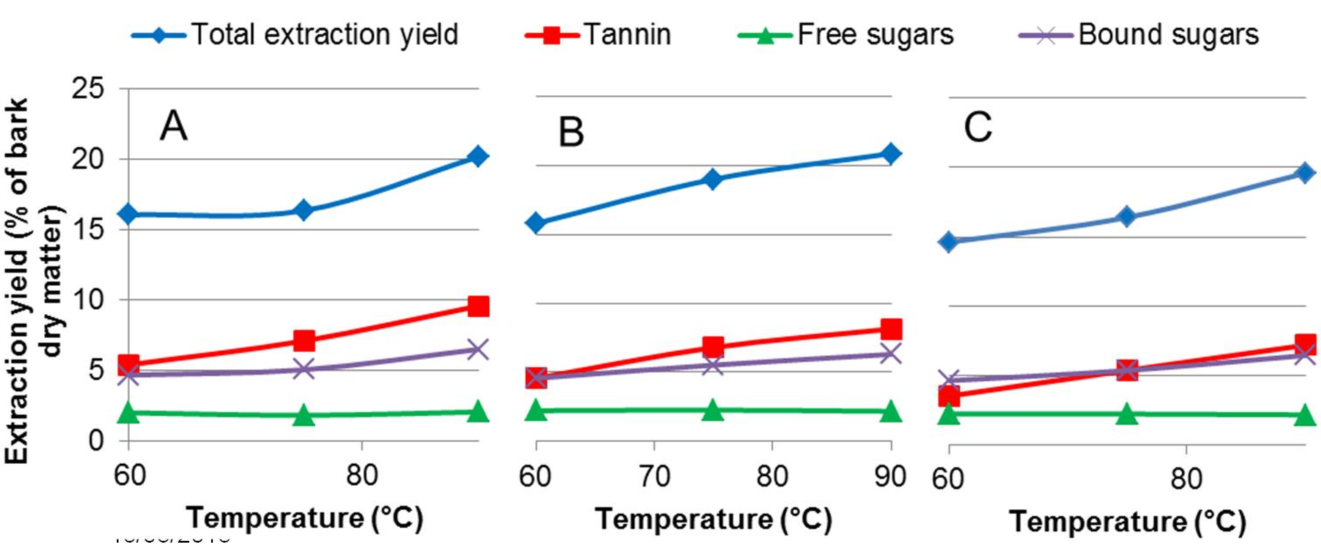
Composition (% of dry matter)	**Fresh bark**	**Aged bark**
Neutral carbohydrates	40.6	33.6
Acidic carbohydrates	6.9	6.5
Lipophilic extractives	2.4	2.3
Acid insoluble lignin	33.5	41.2
Acid soluble lignin	3.3	1.5
Ash	3.1	3.9
Tannin (hide powder method)	10.7	6.3

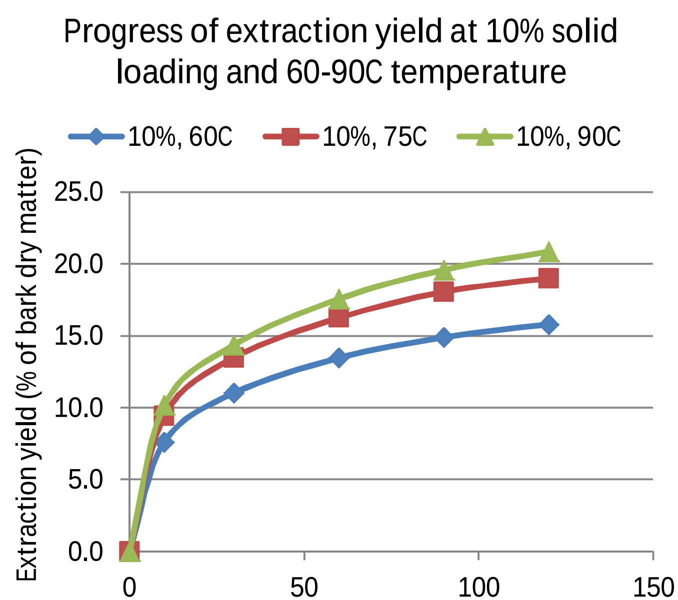
Optimization of hot water extraction in bench scale

- The aim was to find out how extraction temperature, solid-liquid ratio and the use of extraction chemicals affect the solubilization of tannins and carbohydrates.
- Factors
  - Temperature: 60˚C, 75˚C 90˚C
  - Consistency (solid-liquid ratio): 5%, 10%, 15%
  - Extraction chemicals: no chemicals or 2% sodium bisulphite and 0.5% sodium carbonate dosed per bark dry matter
- Procedure
  - Extraction experiments were carried out in a 15 L vessel with 8 kg total mass.
  - The vessel (pressure cooker) rotated around at 2.3 rpm.
  - Extract was collected from a sample valve at 10 min, 30 min, 60 min, 90 min and 120 min and centrifuged before storing in freezer.

Optimization of hot water extraction in bench scale

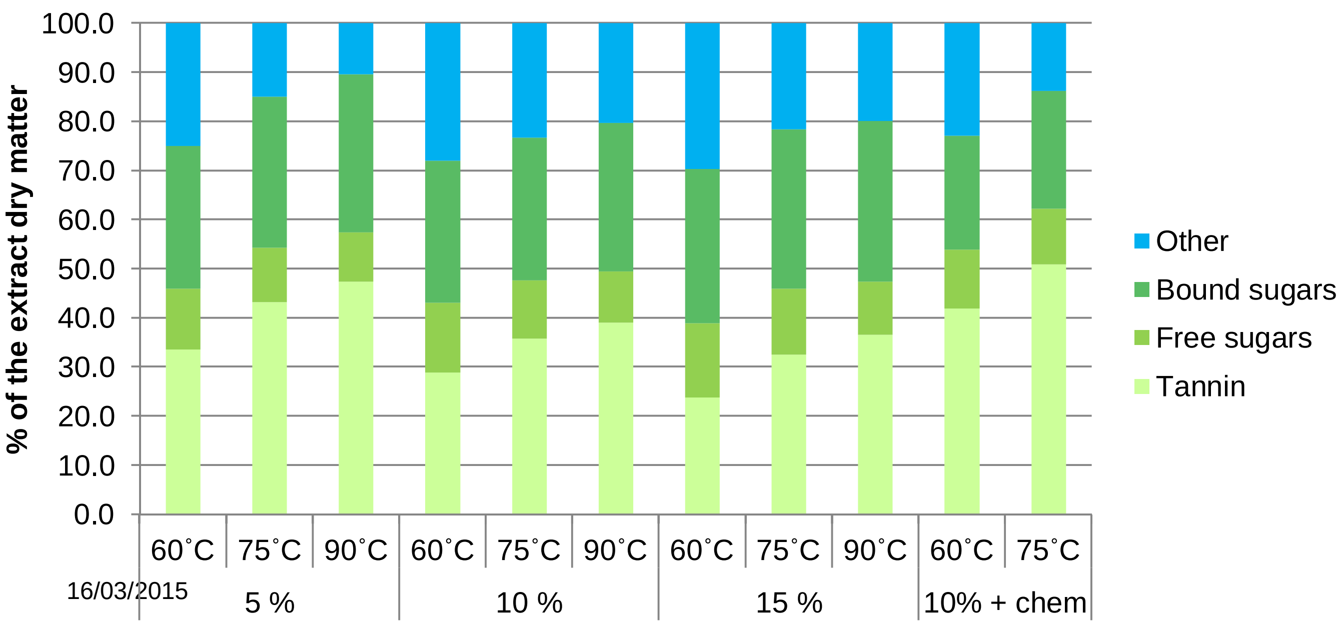
- Up to 20.9% of bark dry matter solubilized during the extraction.
- Based on the development of extraction yield, 120 min was a sufficient extraction time for spruce bark.
- Temperature and solids content affected the extraction yield of tannin and bound sugars similarly, whereas the extraction parameters had little effect on the extraction yield of free monosaccharides.
- A significant part (24-34%) of the sugars in the extract were present as free monosaccharides.

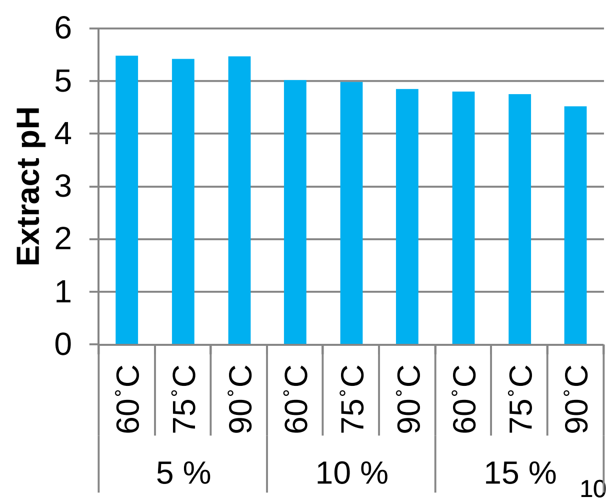




Optimization of hot water extraction in bench scale

- An extract with over 50% tannin content could be produced in bench-scale.
- Extracts contained 35-47% carbohydrates out of which a significant part (24-34%) was present as free monosaccharides.
- Extraction chemicals improved the solubility of tannin and increased the portion of tannins in the extract.
- pH of the extract decreased as a function of the dry matter concentration in the extract.





Pilot-scale extractions of tannin

- Two pilot-scale extractions were carried out in the project, first according to the results of the bench-scale optimization and the second without chemicals as requested by partners working with insulating foams.
- Up to 22 kg of crude tannin extract could be produced with a solubilization yield of 15% and a practical yield of 9% from original bark dry matter.
- The differences in the yields can be explained by the wood content of the raw material and extraction temperature.

Collection and extraction time of the bark	**August 2012**	**June 2013**
Amount of bark extracted (kg, dry)	114	243
Amount of wood impurities in bark (% DM)	21	10
Particle size	Shredded at the sawmill, 1-10 cm	Shredded at the sawmill and crushed, < 1cm
Number of extraction batches	6	3
Reactor volume (L)	250	1800
Extraction set-up	Bark placed in filter bags, forced circulation of the solvent	Bark and solvent mixed freely in the reactor
Extraction temperature (°C)	75	90
Solids content in extraction (%)	9	5
Extraction time (min)	120	120
Extraction chemicals	2% sodium bisulfite, 0.5% sodium carbonate	None
Washing	Once	No
Solubilization yield (%, soluble DM concentration in the extract * water in extraction / original amount of bark)	11.8	14.5
Practical yield (%, crude dried extract / original amount of bark)	8.3	9.1
Amount of crude extract obtained (kg)	9.4	22.1

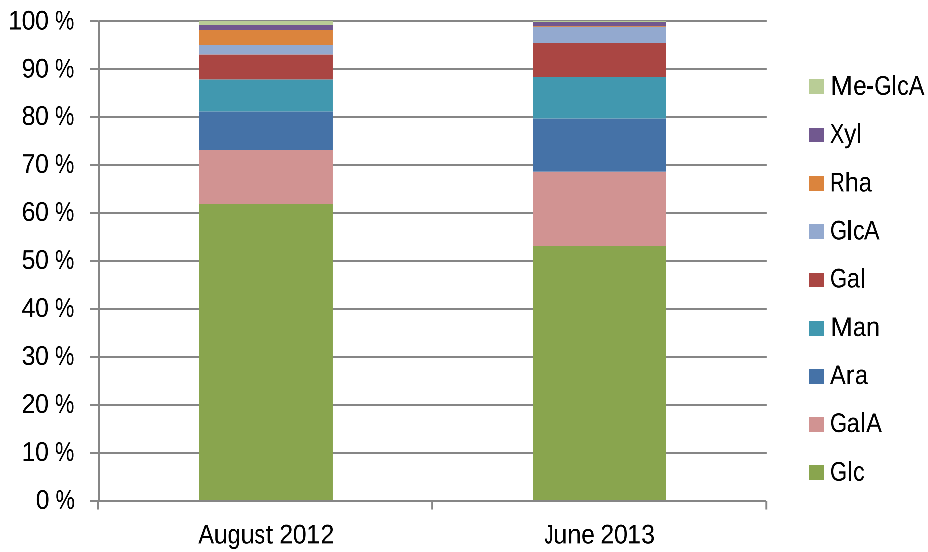
Pilot-scale extractions of tannin

- Both extracts contained at least 50% tannin as analysed by the hide powder method.
- Carbohydrates accounted for up to 30% of the extract dry matter.
- The ash content in the August 2012 extract was significantly higher due to the extraction chemicals sodium bisulfite and sodium carbonate used in the extraction.
- June 2013 extract was not completely water soluble and had a higher content of acid insoluble material. It is suspected that the lack of sulphonation of tannin during the extraction, the higher extraction temperature, and the storage of the extract in cold in concentrated form prior to spray-drying may have caused the formation of insoluble phlobaphenes from condensed tannins.

Pilot-scale extraction	August 2012 (% of DM)	June 2013 (% of DM)
Water-soluble solids	100	80.1
Tannin (hide powder method)	49.7	57.6
Tannin (acid butanol method)	50.5	23.5
Carbohydrates (expressed as monosaccharides)	22.2	30.0
Ash	26.5	4.2
Acid insoluble material	34.5	46.8
Acid soluble lignin	12.3	9.4
Lipophilic extractives	0.9	2.6

Pilot-scale extractions of tannin

- Although the extracts contained different amounts of carbohydrates, their carbohydrate profiles were relatively similar.
- However, June 2013 extract contained less glucose and more galaturonic acid (pectin), arabinose and mannose.
- Share of free monosaccharides out of all carbohydrates in the extracts was less than 8%.

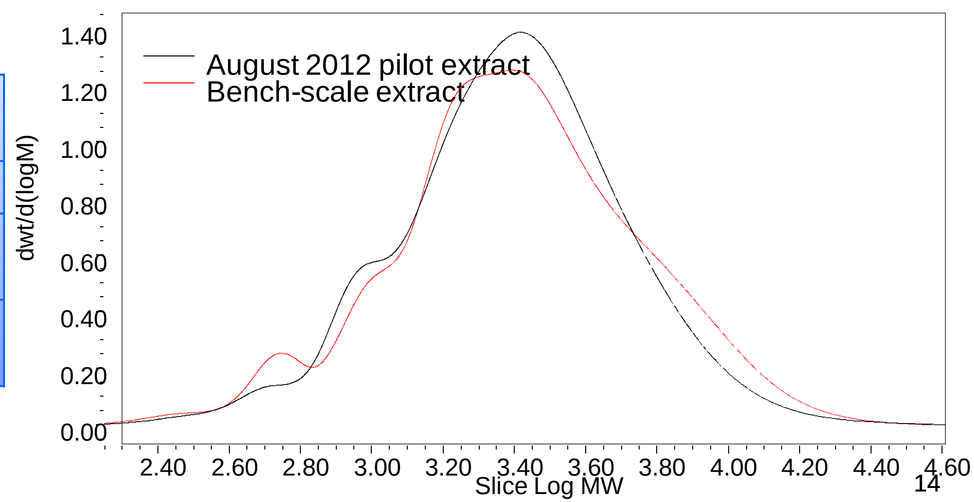


Share of free monosaccharides (% of theoretical)		
	August 2012	June 2013
Ara	7.9	7.9
Gal	1.9	1.2
Glc	6.4	6.2
Man	0.0	0.6
GlcA	5.3	0.0
GalA	16.9	7.9
SUM	7.8	6.7

Pilot-scale extraction of tannin

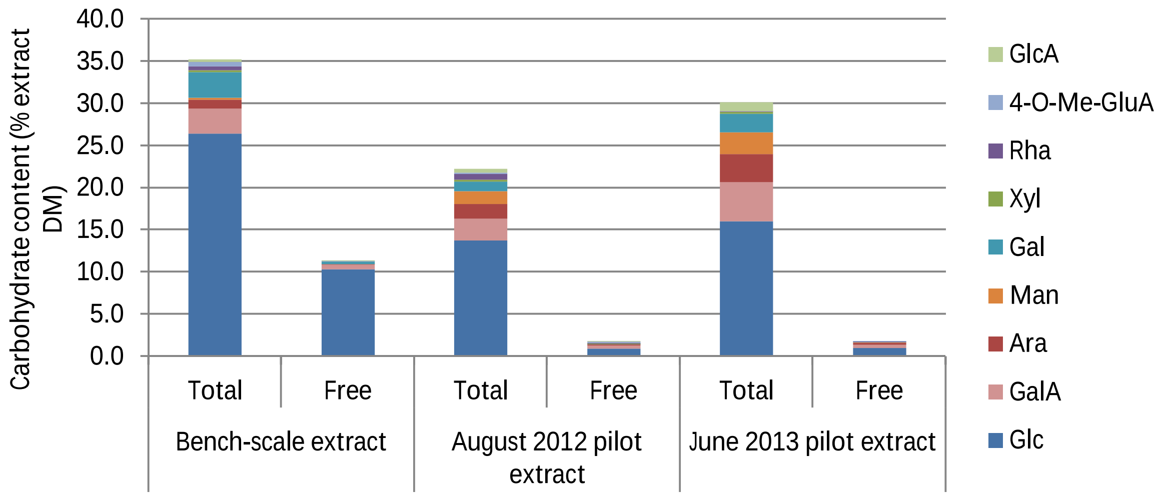
- Several methods were used to characterize the extracts:
  - Folin-Ciocalteau assay showed that the total phenolic content of the extracts was 23-36%, and that the phenolic content was higher in the August 2012 pilot-scale extract compared to the corresponding bench scale extract possibly reflecting seasonal changes in bark composition.
  - Size exclusion chromatography (SEC) analysis showed that the extracts had a weight average molar mass of 3.2-3.4 kDa.
  - ^{31}P NMR analysis showed that the extracts contained several times less phenolic groups than purified quebracho tannin.

Type	Aliphatic OH	Phenolic OH	Carboxylic acid	Total OH
Unit	mmol/g	mmol/g	mmol/g	mmol/g
August 2012 pilot extract	4.22	2.16	0.18	6.56
Purified quebracho tannin	3.99	14.62	0.22	18.83



Purification of crude tannin

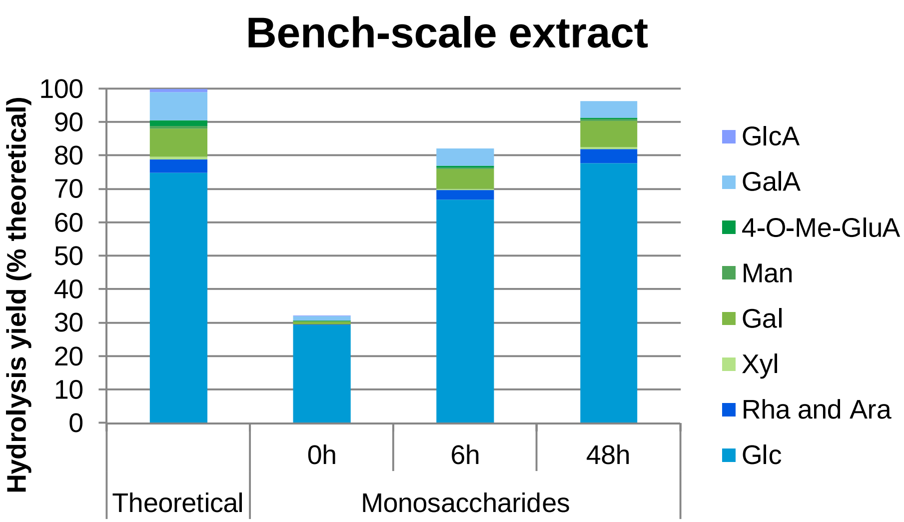
- Target in the development of the purification method was to:
  - Hydrolyse bound carbohydrates in the extract into free monosaccharides using hydrolytic enzymes.
  - Separate the monosaccharides from larger tannins by ultrafiltration.
- Extracts:
  - Bench-scale extract (75°C, 10% solids loading, extraction chemicals)
  - August 2012 pilot-scale extract (75°C, 9% solids loading, extraction chemicals)
  - June 2013 pilot-scale extract (90°C, 5% solids loading, no extraction chemicals)
- Bench-scale extract produced from bark collected in February contained a significant portion of free glucose.

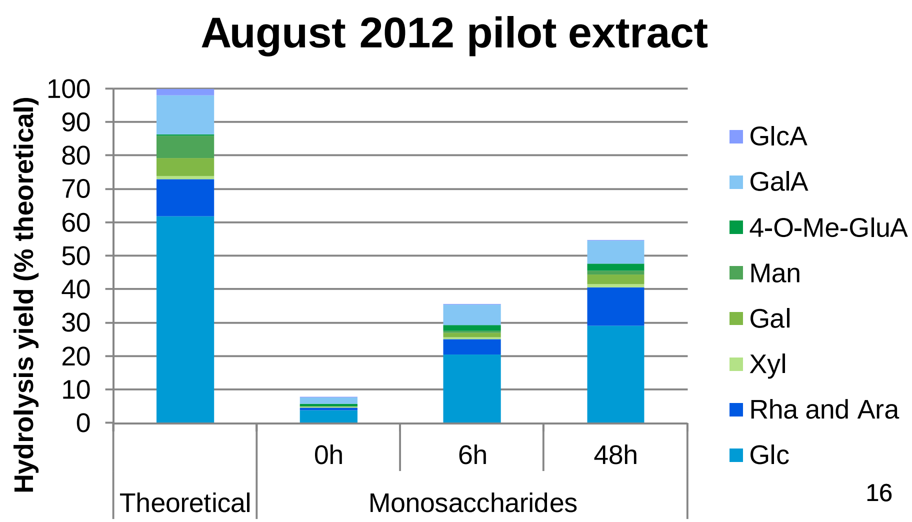


Purification of crude tannin: Hydrolysis

- First, seven commercial enzymes were screened and three were found most potential for the task.
- The mixture of 1/3 Viscozym, 1/3 Novozym 188 and 1/3 Pectinex enzymes was found to be optimal for the bench-scale extract as it hydrolysed close to 100% of the carbohydrates in the extract to monosaccharides.
- The same enzyme mixture could hydrolyse only 55% of the carbohydrates in the corresponding pilot extract from 2012.
- It was suspected that the differences were caused by felling and debarking of the logs in different times of the year (bench-scale bark in winter, pilot-scale bark in the summer).

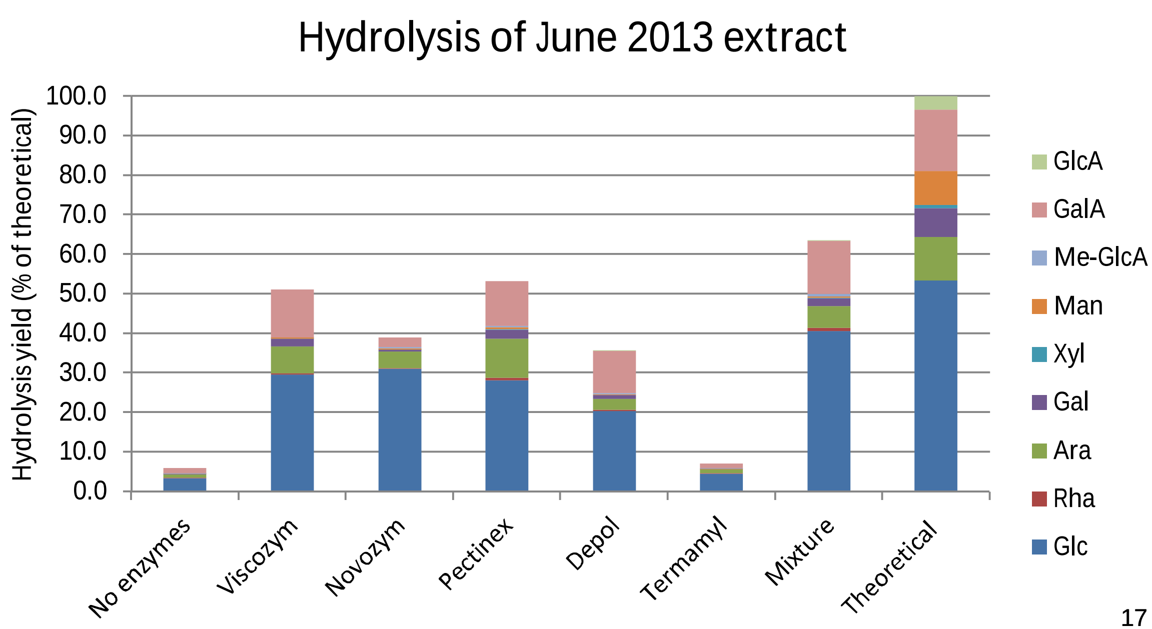
Treatment parameters
- 6-48 h treatment time
- No pH adjustment
- 10 mg/g enzyme dosage





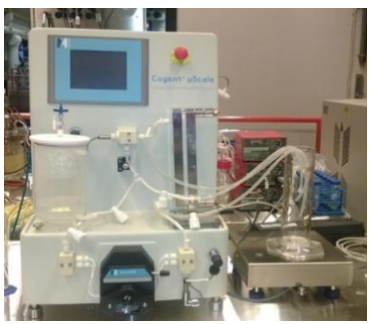
Purification of crude tannin: Hydrolysis

- 19 new enzyme preparations were screened to find enzymes capable of improving the hydrolysis yield on the August 2012 pilot extract.
  - Two more were selected for further experiments, Termamyl containing amylase activity and Depol 40 containing hemicellulase activities.
  - Only slight improvement in the hydrolysis of glucose and arabinose could be obtained with the new enzymes, overall yields remained below 60%.
- June 2013 extract was hydrolysed with individual enzymes and their mixture
  - 64% hydrolysis yield could be reached in 6 h.



Purification of crude tannin: Ultrafiltration

- Preliminary experiments in were carried out in centrifuge tubes and in a dead-end filtration systems.
  - Enzyme treatment slowed down the filtration rate but enabled the removal of 22% of carbohydrates at a cost of 7% tannin loss when a 10 kDa membrane was used.
- More industrially relevant filtration experiments were carried out in three systems:
  - **Lab-scale:** Cogent µScale Tangential Flow Filtration System with 88 cm^2 membrane area and 10 kDa polyethersulfone membrane.
  - **Bench-scale:** Alfa Laval LabStak M20 ultrafiltration unit 0.1 m^2 membrane area and 5 kD polyethersulfone membrane.
  - **Pilot-scale:** Alfa Laval LabStak M39 ultrafiltration unit with 0.8 m^2 membrane area and 5 kDa polyethersulfone membrane.



Purification of crude tannin: Ultrafiltration

- Lab-scale experiments showed that enzymatic treatment increased the filtration rate, but tannins could permeate the 10 kDa memberane too efficiently.
- In bench scale 59% of the carbohydrates could be removed but at a simultaneous loss of 32% of tannin using a 5 kDa membrane.
- In pilot-scale filtration experiments the membrane became soon fouled and the filtration rate reduced significantly. Removal of fine solids from the extract was attempted but the flowrate remained low. Finally the membrane broke and filtration was seized. 26% of carbohydrates could be removed at a loss of 9% tannin.

	Lab	**Bench**	**Pilot**	**Unit**
Membrane pore size	10	5	5	kDa
Rate of filtration	27.3	30		$L/m^2/h$
Hydrolysis yield	56.7	68.9	67.4	%
Monosaccharide removal in filtration	74.0	85.8	38.5	%
Total sugar removal	42.0	59.1	25.9	%
Loss of tannin	31.7	32.1	9.3	%

Purification of crude tannin: Solvent purification

- VTT shortly attempted solvent purification of August 2012 crude tannin by dissolution to three solvents:
  - 94% ethanol
  - 70% ethanol
  - 70% acetone
- Crude tannin dissolved poorly to 94% ethanol but better to 70% ethanol and 70% acetone.
- However, more than half of tannins did not dissolve in the solvent whereas sugars dissolved surprisingly well. The results suggests that tannins and carbohydrates are chemically bound to each other.
- Partner Ledoga purified August 2013 pilot extract using their proprietary solvent-based process and were able to prepare an extract with close to 80% tannin content.

	Tannin content (%)	Neutral sugar content (%)	Total yield (%)	Tannin yield (%)	Sugar yield (%)
Crude tannin	50.5	19.1			
94% ethanol	47.9	18.4	9.8	9.3	9.4
70% ethanol	39.1	18.2	60.0	46.5	57.2
70% acetone	36.3	17.3	64.7	46.5	58.5

Composition of crude and purified tannins

- All extracts contained at least 50% tannins as analysed by the hide powder method.
- Ash content was strogly dependent on the use of extraction chemicals.
- Extracts contained up to 32% carbohydrates. The amount of carbohydrates could be reduced and tannins enriched by Ledoga's solvent purification method. Enzymatic purification procedure did not decrease the carbohydrate content of the extract due to incomplete filtration, and the removal of some insoluble solids, which increased the relative share of carbohydrates in the sample.

Composition (% DM)	June 2012 pilot extract	August 2013 pilot extract	Solvent purified extract	Enzymatically purified extract
Insolubles	0	22.7	0	14.4
Tannins	49.7	57.6	78.8	56.2
Ash	26.5	4.2	3	5.2
Carbohydrates (anhydrous)	22.3	27.0	13.8	32.0
Glc	13.8	14.4	9.9	15.6
Ara	1.8	2.9	2.4	4.2
GalA	2.6	4.2	0.3	3.8
Man	1.5	2.3	0.3	3.6
Gal	1.2	1.9	0.2	2.8
Other	1.5	1.1	0.7	2.1

Conclusions (1/4)

- Spruce bark composition
  - Spruce bark is a promising source of condensed tannins (11-12% tannin). Its composition is different from wood as it contains a significant amount of pectin and non-cellulosic glucose. Carbohydrate profile appears to have slight seasonal variation especially in terms of the amount of free glucose in bark.
  - Dry debarked industrial spruce bark may contain significant amounts of wood as impurities.
- Storage of bark prior to extraction
  - Storage of bark in piles outside lead to a decrease in carbohydrate, acid soluble lignin and tannin content and an increase in the content of acid insoluble lignin. Apparently tannin condensed into insoluble matter (phlobaphenes) which shows up in analysis as acid insoluble lignin. Bark should not be stored but used as fresh as possible after debarking.

Conclusions (2/4)

- Hot water extraction yields and extract composition
  - High temperature increased the extraction yield of both carbohydrates and tannin. It appears that selective extraction of tannins without carbohydrates is not possible.
  - Using extraction chemicals reduced the amount of carbohydrates, increased the amount of tannin and improved the stability of the extract in soluble form. Extracts produced with extraction chemicals contained a significant amount of ash.
  - Spruce bark hot water extracts produced in pilot scale contained 22-30% carbohydrates and circa 50-58% tannin according to the hide powder method. Carbohydrates were mostly in bound form (glycosides, polysaccharides, oligosaccharides), but in winter bark extracts monosaccharides amounted up to 30% of all carbohydrates.

Conclusions (3/4)

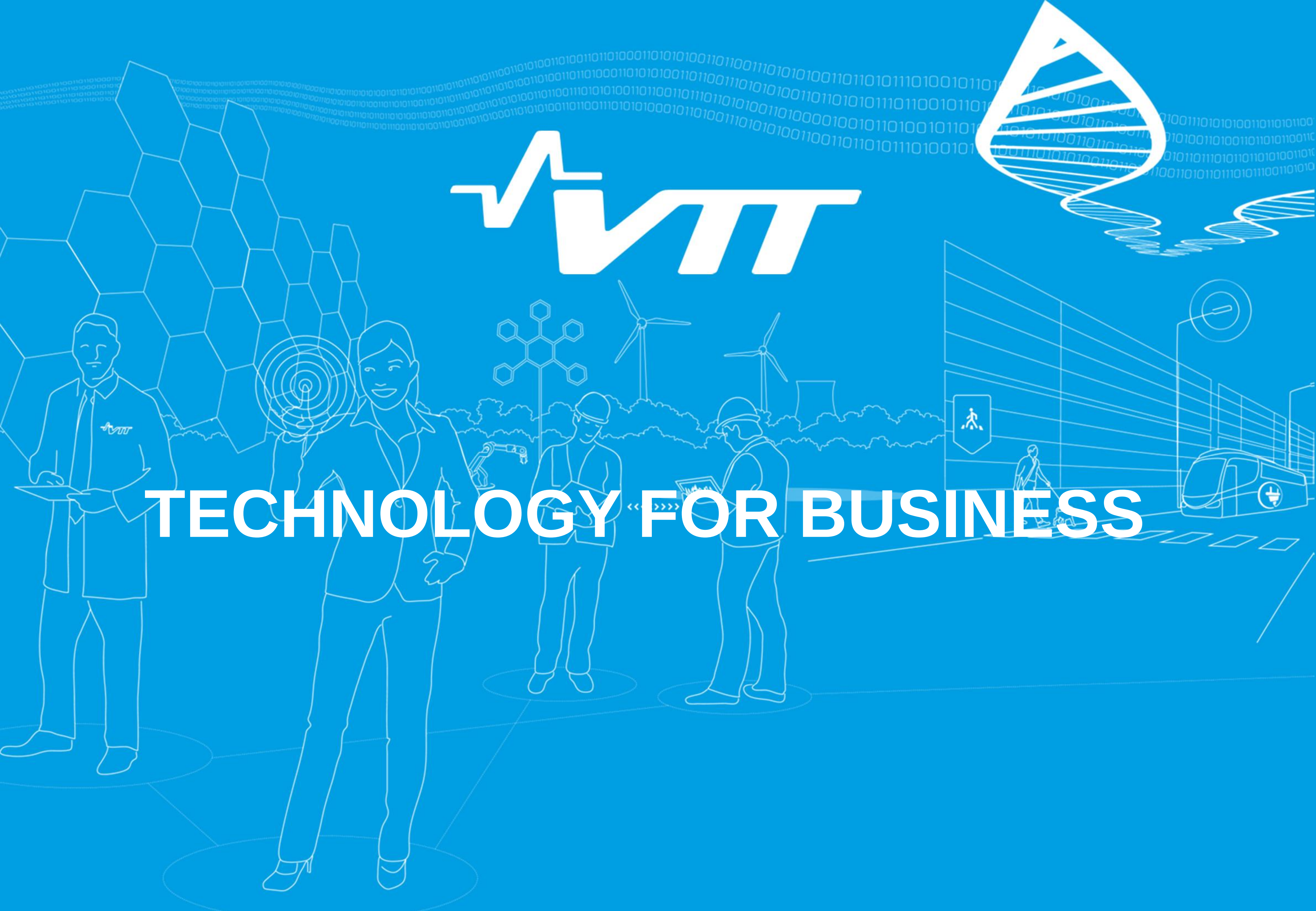
- Purification of hot water extracts
  - Enzymes (betaglucosidases, pectinases, hemicellulases) can be used to hydrolyse bound sugars in the extract to monosaccharides, although the susceptibility of the extract to hydrolysis may have seasonal variation.
  - There is some indication that size-based separation of tannin from monosaccharides by ultrafiltration could be used to purify the extract.
  - Silvateam's proprietary solvent based purification procedure produces an extract with 80% tannin content and a lower carbohydrate content compared to the crude extract.
- Production of spruce bark tannin in pilot scale
  - Up to 22 kg of crude dried extract could be produced at VTT pilot facility with a 9% yield from raw material. With optimal industrial extraction equipment one could reach 12-16% yield.
  - Composition of the extract was similar to those produced in laboratory scale.
  - Spruce bark hot water extract, especially when it has been produced without extraction chemicals, should be dried as soon as possible, as storage in concentrated form may reduce its water solubility.

Conclusions (4/4)

- Industrial extraction of spruce bark (experiences from trials at Ledoga’s plant in Italy)
  - Spruce bark can be chipped and conveyed at the plant using the same equipment as with chestnut wood.
  - Spruce bark cannot be extracted with equipment used for tannin extraction from chestnut wood chips due to higher softness and compressibility of spruce bark leading to increased bridging tendency both before (dry) and after (wet) extraction, and due to the higher water retention capacity of spruce bark.
  - However, it is very likely that commercial equipment suitable for tannin extraction from spruce bark are on the market. Determination of the right equipment needs to be done in cooperation with an engineering company and equipment providers.
  - The project does not provide information on the behaviour of spurce bark extract during concentration and drying in industrial equipment, this needs to be checked in the future.

Dissemination

- Peer-reviewed publications
  - Kemppainen, K., Siika-aho, M., Pattathil, S., Giovando, S., Kruus, K. 2014. Spruce bark as an industrial source of condensed tannins and non-cellulosic sugars. Ind. Crops Prod. 52, 158–168.
  - Lacoste, C., Čop, M., Kemppainen, K., Giovando, S., Pizzi, A., Laborie, M.-P., Sernek, M., Celzard, A., 2014. Biobased foams from condensed tannin extracts from Norway spruce (*Picea abies*) bark. Submitted to Ind. Crops Prod.
- Conference presentations
  - Kemppainen, K., Grönberg, V., Siika-aho, M., Nakari-Setälä, T., Kruus, K., 2014. Valorization of spruce bark. 3rd Symposium on Biotechnology Applied to Lignocelluloses, October 26-29, Conception, Chile.
  - Kemppainen, K., Garcia Marrero, D.E, Grönberg, V., Sernek, M., Čop, M., Laborie, M.-P. 2014. Spruce bark biorefinery. Nordic Wood Biorefinery Conference, March 25-27, Stockholm, Sweden.
  - Kemppainen, K., Niemelä, T., Inkinen, J., Uusitalo, J., Kaijaluoto, S., Sorsamäki, L., Aaltonen, O., Suurnäkki, A., Peltonen, S., Mikkonen, H., Grönberg, V., Kruus, K., Nakari-Setälä, T., Siika-aho, M., 2013. Spruce bark biorefinery. Biomass Conference & Exhibition, June 3-7, Copenhagen, Denmark
- Conference posters
  - Kemppainen, K., Grönberg, V. 2012. Extraction of hemicellulose and condensed tannin from spruce bark. Nordic Wood Biorefinery Conference, October 23-25, Helsinki, Finland.
  - Kemppainen, K., Siika-aho, M., Kruus, K., 2015. Spruce bark carbohydrate profile. Carbohydrate Bioengineering Meeting, May 10-13, Espoo, Finland.
- Master thesis of Taru Niemelä
  - Removal of sugars from tannin-containing bark extract using hydrolytic enzymes and filtration technology, 2013.
- PhD Thesis of Katariina Kemppainen
  - Production of sugars, ethanol and tannin from spruce bark and recovered fibres. To be defended in March 2015.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