Annual report C4F, 2018

The overall progress and major achievements of the research within C4F

We have produced a number of peer-reviewed publications of high quality, recruited new PhD students and successfully obtained a number of research grants associated with the TC4F program in 2018. We have provided new knowledge and information to the general public on novel potential uses of plant oils, proteins and starches. The interest in our research activities has been increased in 2018 with more contacts with industry and society.
The most important example for this is the success in getting the Plant Protein Factory project, supported by Vinnova with 10 millions for two years, in which a protein factory will be established at SLU Alnarp for protein extraction and fractionation from plant green tissues. This project, initially rooted from TC4F, has attracted the most media attention and society interests in 2018. The subproject about fractionation of proteins from the green tissues of plants for food, cosmetics and industrial applications has also attracted good media and public attentions. Moreover, the subproject about the uses of horticultural wastes left in the field after harvest (Fig. 1) is of direct interest for industries and a PhD student is working on this project within C4F. This project has also generated in a new EIP project in collaboration directly with the industry with 50% financial support from the industry.

Fig. 1 Kale field during harvest. To the right unharvested, middle harvested and to the left ploughed part of the kale field. Approximately half of the kale plant is used for human food, rest is becoming waste. This waste could be used to extract bioactive compounds, such as phenolic compounds, and dietary fiber. Photon by Emilia Berndtsson.

Furthermore, the more nutritional type of GM-potato has attracted high attention of media. The finding of the Yin-Yang system has enabled a closer collaboration with Lantmännen for developing nutritional barley for commercial production and generated two patents: US provisional patent application (No. 63/731,282) and an international patent (WO 2018/182493 A1), as well as one SLU Grogrund project. Successful production of human hemoglobin in plants has attracted attention from the food industry. The results from the Problöja resulted in a new Vinnova application to build a pilot factory. A new source of transglutaminase and their different effects on dough and baking quality are important findings in protein research (Ceresino et al. 2018a and b and Fig. 2).

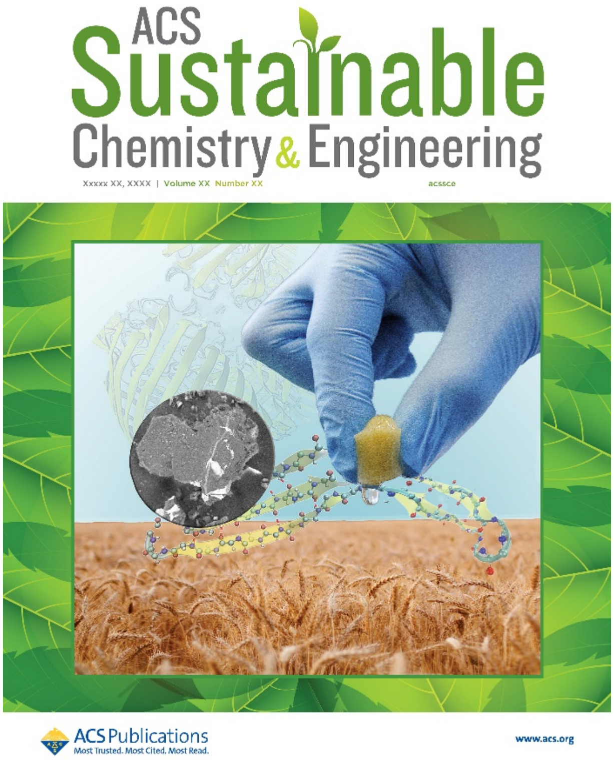

Fig. 2. The use of modified proteins from bioresources raises a sustainable and economical alternative to water-absorbent materials. These materials with high swelling capability have been prepared as particles and foams, both resembling the swelling properties of their petroleum-based counterparts." Volume 7, Issue 5, Pages 4532-5587 https://pubs.acs.org/toc/ascecg/7/5

More research findings and progress

In the biobased material part, we have published the work on resistant starch (RS) in high amylose potato, in which we concluded that the elongated outer chains of the amylopectin in the high-amylose potato gives recrystallized amylopectin that is partly resistant (Zhao et al 2018). Developing a simplified method for determination of the branching density in amylopectin has been in good progress.

We have recently found that the higher oil content in wild rice is regulated by a single gene and overexpression of this gene in a rice cultivar (Fig. 3) led to significantly high oil content and biotic stress tolerance against rice brown plant hopper and rice blast fungus.

Fig. 3 Transgenic rice plants overexpressing a lipid biosynthetic gene showing increased oil content. Photo by Chuanxin Sun.

We have finalized and submitted three manuscripts in carbon allocation: **1)** phenotypic, metabolic and transcriptomic implications of oil production in wheat endosperm overexpressing oat *WRI1*; 2**)** analysis and comparison of transcription factor gene expression of oat embryo and endosperm; **3)** functions of the transcription factor *WRI1* in channeling carbon to oil in relation to structures and functions of different protein subdomains as well as autoregulation between the WRI1 protein and its cis-controlling elements. A large set of RNAseq data regarding transcription factors (TFs) involved in embryo development and oil accumulation were obtained and some TFs will be validated regarding their interactions in oil accumulation.

The different isoforms of crambe DGATs, the final enzyme in oil accumulation, have been further studied with focus on the fatty acid 22:1 as substrate for further increasing its level in seed oil. The combinatorial effects of the acyl donor and acyl acceptor for the DGATs have been investigated and the result shows that the crambe DGAT isoforms readily accept 22:1-CoA as acyl donor, but inefficient in utilizing di-22:1-DAG as acyl acceptor.

We have successfully developed an efficient protocol for protoplast isolation and growth as well as callus proliferation of *Lepidium campestre* (Fig. 4), which is essential for genome editing using CRISPR/Cas9 for production of mutation lines without any external DNA integration into the plant genome.

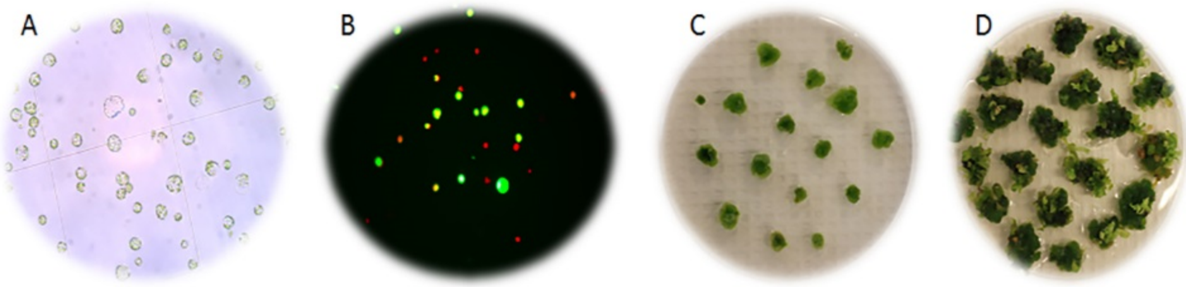

Fig. 4 Protoplast regeneration process of *Lepidium campestre*. A: Freshly isolated protoplasts. B: Protoplast viability test by FDA, green protoplasts are living ones. C: Callus formation from protoplasts. D: Shoot formation from the callus. Photos by Emelie Ivarson, Sjur Sandgrind and Li-Hua Zhu.

Field trials on camelica (Fig. 5) were conducted in Nebraska and Sweden and seeds were collected for analyzing the target pheromone precursor content. A new compound for pheromone production and wax esters have been produced in camelina seed oil that could potentially be used as pheromone precursors.

Fig. 5 Camelina (*Camelina sativa*) plants in the field trial in Borgeby that are used for extraction of oil and wax esters for production of insect pheromones, which are then used for control of insects in more secure and environmentally friendly manner than what are used today. Photo by Per Hofvander.

We have established 1) Raspberry Pi–controlled imaging platform for monitoring kinetics of seed germination and early seedling growth that enables accurate comparison among genotypes and sampling for downstream lipid analysis; 2) established chemical screen and validation pipeline for discovery of small molecules with autophagy modulating activity in plants. Three new activators and one inhibitor of autophagy have been identified. We have also performed transcriptional profiling of *ATG* genes in Arabidopsis during starvation-induced autophagy response with the ultimate goal to discover transcription factors controlling rate-limiting steps of autophagy.

Scientific publications (peer reviewed articles and book chapters)

Andersson, M., **Turesson, H.**, Olsson, N., **Fält, A-S**., Olsson, P., Gonzalez, M., Samuelsson, **Per Hofvander, P.** 2018. Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery. Physiologia Plantarum, 164: 378-384.

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Muneer F, Johansson E, Hedenqvist MS, Plivelic TS, Markedal KE, Petersen IL, Sorensen JC, **Kuktaite R** (2018) The impact of newly produced protein and dietary fiber rich fractions of yellow pea (*Pisum sativum* L.) on the structure and mechanical properties of pasta-like sheets. Food Res Int. 106:607-618.

Muneer F, Johansson E, Hedenqvist MS, Plivelic TS, **Kuktaite R (2018)** Impact of pH-mediated modification on protein molecular profile and structure-function relationship in potato protein-wheat gluten based biocomposites. Int. J. Mol. Sci. XXXXXXX

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Diuk Andrade F, **Newson WR**, Bernardinelli OD, **Rasheed F**, Cobo MF, Plivelic TS, Ribeiro deAzevedo E, **Kuktaite R**. An insight into molecular motions and phase composition of gliadin/glutenin glycerol blends studied by 13C solid-state and 1H time-domain NMR. Journal of Polymer Science Part B: Polymer Physics. 2018 May 1;56: 739-50.

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