Gene-edited wheat
Time for Coeliac sufferers to rejoice but at what cost?

ALSO INSIDE:

Innovation, innovation, innovation
From universities to suppliers – it’s all over this issue like a rash

Cultured plant cells
The solution to global resources

How fluffy are your cakes?
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Gene-edited wheat

Gene-edited wheat: New hope for Coeliac sufferers?

P12 Cultured plant cells

Plant cell culture is the new buzzword at VTT labs. Global population expansion, environmental issues, reducing arable land means serious supply issues. Plant cell culture offers a possible lifeline.

P18 Cake fluffiness – perfection is mathematically possible!

If you thought school maths was of no use in the commercial world, look away now! Campden BRI explains how to achieve the ideal fluffiness of your cakes using graphs and equations.

P21 Health and sustainability conference – Campden BRI

A preview of this conference that will debate nutritional value and sustainable issues with keynote presentations from leading professionals from industry, research and academia.

Spaces are limited!

P26 Bread roll plant – waste reduction

Prevention and removal of excess sticky dough from surfaces/ pans – researchers at the University of Birmingham have been working hard in the background to resolve this wasteful and costly problem. Read on to see if you too can save some dough!

P42 From across the pond

Colourful bakery products

In 2019 the colour of your bakery products matters - greatly, according to research from Penn State University, USA – and the darker/more colourful the better - even purple bread is seen as healthy.

P46 Hovis® is going green.

Fortunately, it is their new electric vehicles and not their bread that is green! Timed perfectly with the introduction of London’s new Ultra-Low Emission Zone (April 2019), the 130-year-old bakery befriends the environment.

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Many of us in Europe associate this time of the year with spring cleaning, new ideas, a fresh coat of paint etc. The industrial bakery industry is in many ways no different in that it is constantly searching for new ways to feed the ever-changing and sometimes fickle consumer trends. In short, this means innovation in areas such as gluten-free, clean-label, product design and even colour, many of which form the theme of this issue.

Our researchers at Baking Europe have been busy digging deep in recent weeks to root out some of the exciting cutting-edge research and innovation being carried in Europe’s leading academic, professional and commercial organisations and we kick off with our Cutting-Edge section with a piece from Wageningen university on gene edited wheat which will create Coeliac-safe bread flour. Controversial or socially acceptable you may ask?

Continuing the innovation theme, VTT is deep into research on the use of plant cell culture (PCCs) and alternatives to vegetable sources amidst severe global resource issues. Campden BRI meanwhile, reports on some great results from their labs in which they developed fluffier cakes; using maths and equations, so maybe it’s time to dig out the old school log book and slide rule!

Our USA friends at Penn State University bring some new ideas on using colour in bakery whilst Hovis® have launched their new “green” electric delivery vehicles just in time for the TfL (Transport for London’s) launch of the Ultra-low-emission-zone (ULEZ) introduced in April this year and saving them £12.50 a day per vehicle and contributing to the reduction of the city’s high pollution levels.

Baking Europe’s commercial partners have also been bitten by the innovation bug! (There must be something in the water!)

We are delighted to present Innovation Profiles from Stable Micro Systems on resolving salt reduction/stickiness problems in dough, Koenig on bun line planning and installation, VMI joining forces with ONIRIS on new developments in mixing geometry and last, but not least, Rademaker on a new bread line.

Oh, and please don’t forget all our other advertisers who support us – give them a quick call. You never know, they may also have something new that could revolutionise your bakery system.

And finally, with innovation being the theme, our quote of this issue has to go to Sir Winston Churchill who said:

“Without tradition, art is a flock of sheep without a shepherd. Without innovation, it is a corpse”

Graham Pendred
Publisher
Gluten proteins from wheat form the gas-holding network in a dough enabling the baking of an attractive high-volume bread. However, these proteins also contain peptide fragments that may cause coeliac disease (gluten intolerance) in genetically predisposed individuals. About one to two percent of the population suffers from this disease, which causes an inflammation of the small intestine. A strict, lifelong gluten-free diet is the only remedy, which in practice is difficult to comply with because wheat and (isolated, ‘vital’) gluten have numerous food-technological applications (e.g. as binder) in a wide variety of food products beyond bakery. Many strategies have been considered to make wheat-containing food products coeliac-safe through food processing technologies (including grain malting, sourdough fermentation, peptidase application or gluten substitution), or through plant breeding (including variety selection, mutation breeding, and gene-silencing) (Gilissen et al., 2014; Jouanin et al., 2018a). However, most of these strategies have limited success. Some are promising or challenging; the application of CRISPR/Cas (clustered regularly interspaced short palindromic repeats and CRISPR-associated protein) as an advanced breeding tool belongs to this category and will be discussed below.

A breeding strategy towards hypoimmunogenic or coeliac-safe wheat requires a complete and detailed knowledge of the complex set of gluten genes and the proteins they code for. The number of gluten genes may vary among the different wheat species and varieties. A
As a proof of principle, wheat plants have been generated in which some gliadin genes have been edited or removed successfully... these plants are not yet safe for coeliac patients.
recent analysis of the genome sequence of the hexaploid variety ‘Chinese Spring’ (International Wheat Genome Sequencing Consortium et al. 2018) gave a reliable indication of the numbers of the genes per gene families: 45 alpha-gliadins, 18 gamma-gliadins and 10 omega-gliadin genes, next to 6 high molecular weight (HMW) and 16 low molecular weight (LMW) glutenin genes were annotated (Clavajo et al., 2017; Huo et al., 2018; Smulders et al., 2019). Note Gene-edited wheat varieties with safe gluten, therefore, will require the development of novel assays based on the direct quantification of coeliac-inducing epitopes themselves.
that (1) each gene family evolved from a single ancestor gene and gene family members have a high DNA sequence similarity; (2) bread wheat varieties are hexaploid (carrying three independent diploid genomes of 7 chromosomes per genome and (3) especially the alpha and gamma-gliadins carry the major immunogenic fragments (coeliac-inducing epitopes). For coeliac-safe wheat production, the focus has been on these two types of gliadins, but omega-gliadins will need to be tackled as well.

Breeding wheat lines free from coeliac-inducing epitopes would be a definitive solution. Recently, the CRISPR/Cas technology became available for application in gene editing of higher organisms, e.g. to edit gluten genes in wheat. This technology works as follows: a so-called single stranded guide RNA (sgRNA) is designed to be complementary to a gliadin target DNA sequence. This designed sequence may be directed to a sequence common to all gene family members, or to a sequence present in a subset of the genes, such as an epitope sequence. Next, a DNA construct coding for the sgRNA and the Cas endonuclease, a DNA-cutting enzyme (in this case Cas9), is introduced in an immature wheat embryo cell. The sgRNA directs the Cas endonuclease to the target DNA site where it creates a double stranded DNA
The daily struggle of coeliac patients to get safe foods or to avoid unintended consumption of gluten-containing foods is real and substantial.

break. Broken DNA needs to be repaired. The innate repair system of the plant cell may make mistakes, mostly in the form of changes, deletions or insertions of single nucleotides, but also deletion of a part of a single gene or even deletion of a complete set of tandemly arranged homologous genes from a gene family is possible (Figure 1 from Jouanin, 2019; adapted from GenScript, Nanjing, China). The DNA construct coding for the CRISPR sgRNA and the Cas9 protein can be simply eliminated from the progeny of the edited plant by crossing. Screening of the offspring enables the selection of plants with edited target genes but free from the DNA construct.

As a proof of principle, wheat plants have been generated in which some gliadin genes have been edited or removed successfully (Sánchez-León et al. 2018; Jouanin 2019); these plants are not yet safe for coeliac patients. To determine which genes have been targeted in a large number of independent mutated wheat lines, high-throughput screening systems have been developed. Thousands of lines can be screened accurately when using the droplet digital PCR method (Jouanin et al., 2019) and subsequently tens of lines can be re-sequenced after capturing all gluten genes with a DNA capture system, GlutEnSeq (‘exome-capture next-generation Illumina sequence technology’). In this way, the mutated wheat lines, their offspring and lines from subsequent crossings can be screened rapidly and sensitively. Based on the data obtained from these screenings, individual plants can be characterised on the epitope status of remaining gliadin genes that, if needed, still require further mutation in a next CRISPR/Cas treatment.

The use of CRISPR/Cas technology raises interesting technological, legal and ethical topics, not only in plant breeding but also for animal breeding and in medical applications in humans (e.g. to cure genetic diseases). Related to wheat, the following may become relevant.

- **Gluten detection.** Gluten-free food products are analysed with monoclonal antibody assays (such as the R5, the G12 or the Gluten-Tec test) for their total gluten level being below the legally agreed 20 ppm threshold level. In the case of successful CRISPR/Cas edits, safe gluten will remain present in large amounts (with advantages regarding baking quality) which will be quantified far above the 20 ppm level by the antibody assays. Gene-edited wheat varieties with safe gluten, therefore, will require the development of novel assays based on the direct quantification of coeliac-inducing epitopes themselves. This also requires a reconsideration of the term ‘gluten-free’ for coeliac-safe food products.
- **GMO regulation.** Mutation breeding of plants using ionizing irradiation or chemical treatment is commonly accepted and exempted from GM regulation, although these methods are inaccurate and require extended selection
and back-crossing to achieve the ultimately desired plant genotype/phenotype. Mutagenesis by CRISPR/Cas on the other hand is precise and targeted, without significant off-target effects. In the case of CRISPR/Cas, a DNA construct has to be introduced into the cell nucleus transiently or integrated into the genome but later removed through crossing. However, because the process of CRISPR/Cas employs a protocol also used in ‘conventional’ genetic modification, it has been subjected to GM regulation according the ruling of the European Court of Justice in July 2018, even if the product has no introduced genes and may be identical to a natural mutation. Regulation of gene editing as GM will, therefore, impede innovation, competitiveness and access to healthier food in Europe (Jouanin et al. 2018b). Here, the voice of the consumer, in casu the coeliac patients as prominent stakeholders, should be heard (Gilissen and Van den Broeck, 2018).

- Quality of life. The daily struggle of coeliac patients to get safe foods or to avoid unintended consumption of gluten-containing foods is real and substantial. Wheat with safe gluten has the potential to become a serious improvement of the quality of life of these patients. This sounds easy but has some consequences. In the beginning, the cultivation, harvesting, transport, etc. of CRISPR/Cas-mutated wheat should be managed completely separated from regular wheat; processing and production of foods with safe gluten require separate production lines and strict regulation is needed concerning packaging and easy-to-understand, unambiguous ingredient information on the product label. This may change later on, if gene-edited wheat with coeliac-safe gluten would become mainstream.

These and other items can be considered achievable challenges as an integrated part of the CRISPR/Cas-involved coeliac-safe food technological and economic innovation and evolution.

REFERENCES


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Cultured plant cells for human food and nutrition

By Dr Liisa Nohynek and Dr Heiko Rischer

NEW WAYS FOR PLANT-BASED FOOD PRODUCTION NEEDED
For breakfast we want to have healthy berries with our yoghurt, for lunch it would be nice to eat fresh salad with tomatoes, avocado and peanuts and for the dessert, sweet fruits such as banana and mango or just a chocolate bar. Plants and plant-based products are the vital element in our everyday diet. However, increasing global issues including population growth, climate change, contamination of the environment and armed conflicts present severe threats for many agricultural products. Due to the lack of arable land that is required to ensure global food production in the near future, ambitious research is needed to develop alternative technologies in order to meet increasing global demand for healthy plant-based food and nutrients. Plant cell culture, first introduced more than 100 years ago that has since developed continually, is an attractive technology to be considered when resolving the problems of global food production.

WHAT IS PLANT CELL CULTURE (PCC)?
Plant cell cultures consist of individual, undifferentiated cells that can in principle be derived from almost any plant. PCCs consist of so-called totipotent cells containing all genetic information for the development of a functional plant, the production of primary metabolites e.g. proteins, carbohydrates and lipids, as well as secondary metabolites, such as pigments. PCCs are propagated from pieces of wild or cultivated plants or their fruits of which only tiny pieces of plant material are required for initiation of the culture. Plants naturally protect themselves by developing wound or scar tissue coined a ‘callus’ when injured and it is this process which is exploited by PCC technology. Microbe-free plant pieces are kept on simple...
In general the flavour of PCCs is fresh and mild without off-flavours.

nutrient media containing sugar, minerals, a gelling agent and natural plant growth regulators favouring the production of callus on the cuttings. Callus consists of identical cells multiplying continuously, therefore, producing a biomass of cells. These plant cell clumps need regular transfer to a fresh medium in order to maintain their viability and to generate suspension cell cultures once introduced to the media without a gelling agent. The size of a single cell ranges approximately from 20µm to 100µm, their shape being round or oval and small aggregates or chains of these cells may be formed. In a suspension culture, the duration of cultivation of a single batch ranges from a few days to three weeks depending on the plant cell line. The yield of fresh cells varies between 100–650 g/litre of the culture.

PCC technology requires in-depth knowledge of plant physiology and biochemistry combined with the controlled production processes of plant cells. VTT has circa 30 years’ experience in the establishment of plant cell cultures, which were recently included in the VTT culture collection (http://culturecollection.vtt.fi/). To the best of our knowledge there are very few public culture collections containing plant cell cultures worldwide. Nordic plants and in particular wild berries such as cloudberry, bilberry, lingonberry, crowberry and arctic

Figure 2: Plant cells for food
bramble have been of special interest for us and constitute an outstanding resource for novel cell cultures.

Chemically, plant cells resemble those of the original plant and have the potential for production of all natural primary and secondary metabolites (Suvanto et al. 2017). However, the chemical composition of plant cells is not identical with the original plant and edible parts of it, such as berries, fruits or leaves. For instance, a fatty acid composition of cloudberry (Rubus chamaemorus) cell culture more closely resembles the berry seeds, while phenolic compounds detected in the cells are known as building blocks for ellagitannins commonly found in cloudberry leaves and fruits (Nohynek et al. 2014). The spectrum of compounds produced by plant cell cultures can be further adjusted by the selection of cultivation parameters, e.g. illumination, medium components and temperature. For example, illumination increases the intensity and variation of the colours in the cell cultures, such as bright yellow, green and red. PCCs cultivated in darkness are often pale yellow, white or grey containing no pigments, but otherwise the chemical composition is usually similar to the same culture growing under light.

PCC technology may exploit rare plant species and, therefore, protect endangered plants from over-harvesting.
BENEFICIAL PROPERTIES OF PLANT CELLS

Plant cells constitute a totally novel material for global food and nutrition management. The nutritional value of PCCs is excellent and includes proteins, fatty acids, carbohydrates, vitamins, minerals and dietary fibre (Nohynek et al. 2014; Nordlund et al. 2018). However, there are clear differences in these major nutrients between plant species; examples being that cloudberry cells contain a protein content of ca. 20% and lingon cells 13.7%. The sensory characteristics are impacted by the structure of the cell culture as well as the plant species, but in general the flavour of PCCs is fresh and mild without off-flavours (Nordlund et al. 2018).

Colourful plant cell cultures are also a feast for the eye, appearing in bright yellow, orange, green and different shades of red, pink and purple. The reddish colours originate from anthocyanins and yellowish from various carotenoids, which are compounds well known to promote human health. In addition, some plant cell cultures are known to exhibit antimicrobial and antioxidant activities with special relevance for human welfare and shelf-life of the food.

Industrial applications of PCC technology have existed for several decades covering coloured components and cell fractions for cosmetics, pigments for food ingredients and secondary metabolites for pharmaceuticals. PCC products have usually been extracted from the plant cell biomass for these products. Due to the considerable biological properties of PCC-derived compounds there has been a remarkable increase in the various markets. The cosmetic industry in particular, is exploiting current developments and is using plant cell extracts originating from rare or exotic species which also exhibit beneficial properties and bioactivities for human skin health (Trehan et al. 2017).

PRODUCTION OF PLANT CELLS

In the laboratory, plant cells are maintained either on solid media or in shake flasks as suspension cultures and possess the capacity for scaling up to suit industrial applications. The largest known bioreactor volumes for plant cell cultivation currently stand at 75,000 litres and are generally used for cultivation of Taxus cells as an example. The majority of the global demand for the anti-cancer drug paclitaxel is produced in this way (https://phytonbiotech.com/about-pcf/). Both the cultivation and harvesting processes of plant cell cultures at experimental and industrial levels are well controlled resulting in chemically and microbiologically safe plant products. The final product of the PCC process may be a result of the entire culture i.e. a combination of both cells and growth medium, or the cells being separated from the culture by filtration or centrifugal process. The fresh cells are used as they are, or alternatively frozen. In addition, freeze-drying of filtered cells is common practice to obtain cell powder with good stability for industrial applications, such as cosmetics (Nohynek et al. 2014).

There are several advantages in PCC technology. The production of plant cells in fermenter-type
bioreactors is a sustainable process, operational on a year-round basis and independent of the location of the facility. The industry ensures constant availability of plant raw material, manufactures in close proximity to the customer, such as those in cities, ensuring that the consistency and high quality of batch production is guaranteed. The product is free from harmful pollutants and environmentally damaging chemicals such as pesticides, herbicides and contaminants of microbial and animal origin. In addition, PCC technology may exploit rare plant species and, therefore, protect endangered plants from over-harvesting.

**PLANT CELLS FOR FOOD**

It is anticipated that in the near future, the emphasis on food manufacture will shift markedly towards the nutritional value of food rather than quantity and sufficiency.

The benefits of using of plant cells in food manufacture are various, since they are relatively rich in nutrients with compounds that promote human health and the production process has significant environmental and safety advantages. In addition, qualified facilities with adequate know-how already exist for the production of plant cells that are used as food ingredients for a variety of products such as baked goods, muesli, snacks, yoghurts, smoothies, ice creams and jams.

The applicability of PCC technology for human nutrition has recently raised notable interest both in the scientific community (Davies and Delores, 2014; Eibl et al. 2018, Nordlund et al. 2018) and in society in general as indicated by publications of food related news (Fremont, 2017; Science Daily 2018). It has to be said, however, that before PCC-containing products are allowed to enter the market, regulatory issues need to be clarified and the safety of the selected plant cell culture for food products must be confirmed (Murthy et al. 2015). In the EU, use of plant cell culture in

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**REFERENCES**


