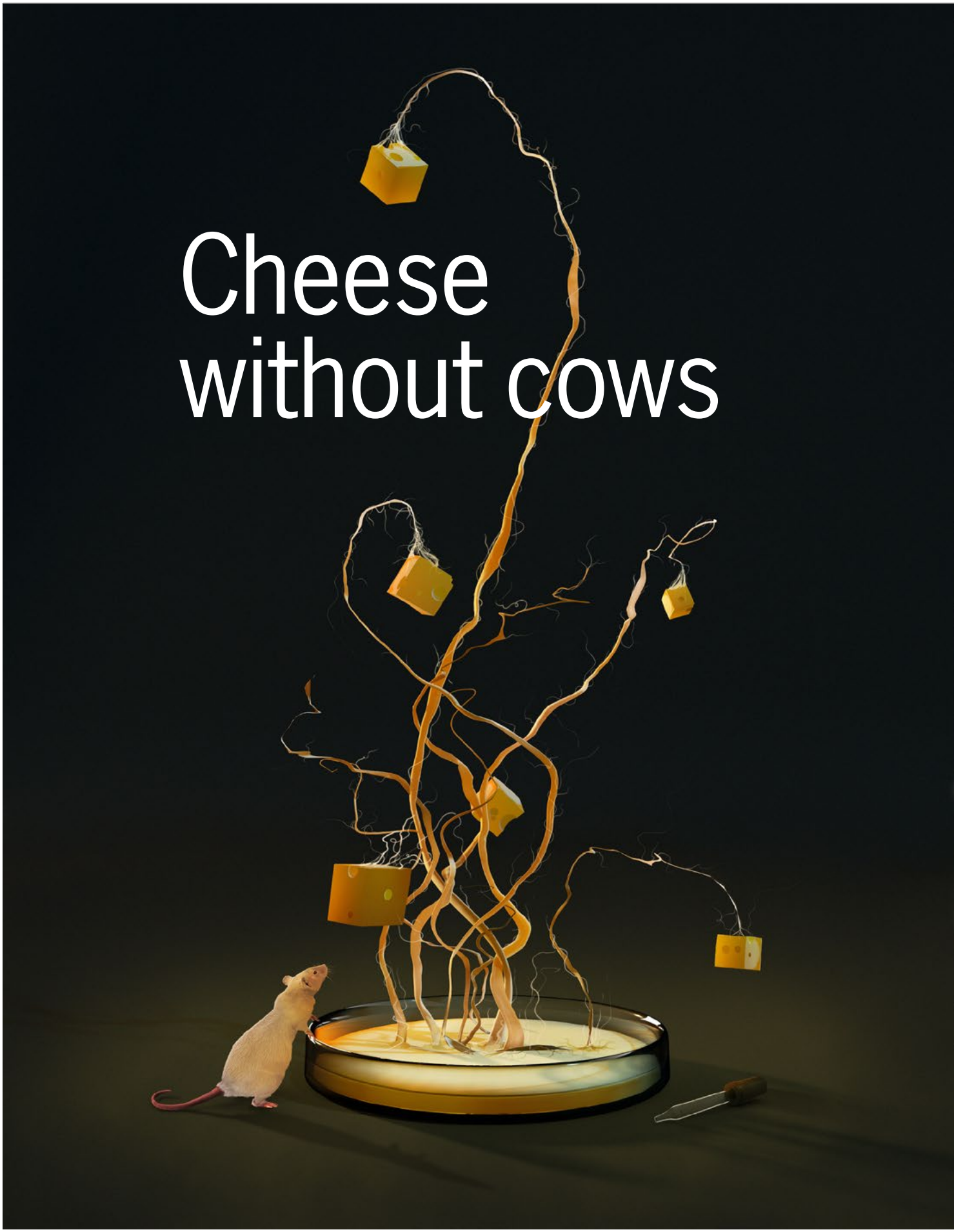
Cheese without cows



Researchers are trying to get micro-organisms to make milk protein. The idea is that this will result in cheese that tastes the same as traditional varieties, but without the use of cows, thus using less land and water and emitting much less CO_2.

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When you pick up a kilo of cheese, you are actually holding six to ten litres of milk. That invisible quantity of milk is why cheese is up there with chicken and pork in terms of its environmental impact. This is largely due to emissions by cows of the powerful greenhouse gas methane. Protein-rich cheese substitutes can provide a sustainable alternative, but the existing vegan cheese products still leave a lot to be desired in terms of flavour and texture.

Anyone aiming to produce a cheese substitute that smells, feels and tastes like traditional cheese should look for proteins that behave exactly like milk protein in the cheese-making process. One option is genetic modification: put the milk protein gene from a cow into a micro-organism, culture the organism in a heated stainless-steel vat and harvest the protein from the culture medium.

'A fair amount of research was being done on making milk protein in micro-organisms as far back as the 1970s,' says Julia Keppler, who works at the Laboratory of Food Process Engineering in Wageningen. 'But that turned out to be very expensive and not feasible on a large scale. Culturing technology has become more affordable over the past ten years, though.'

And Keppler has also gained personal experience of the possibilities for making milk protein by this method. Three years ago, she and her fellow researchers put a cow's milk gene into a bacterium (*E. coli*), and could then extract protein that is almost indistinguishable at the molecular level from the same ingredient in cow's milk. 'It was really an initial demonstration project to show in the lab that the technology works and that the protein is identical. *E. coli* is not an ideal host for making proteins that you want to use as a food ingredient, because some strains of this species of bacteria cause infections in humans.'

Keppler is currently working within an EU project focusing on using an animal-free version of the milk protein casein for cheese making, and is leader of the Protein Transition 2.0 consortium on the purification and application of animal-free milk proteins, which has received 1.9 million euros as part of the Dutch Research Council's Key Enabling Technologies Agenda.

CELLULAR AGRICULTURE

The technology for producing animal products such as milk and meat directly from cells, referred to as 'cellular agriculture', has been coming in for more attention over the past two years. This is partly thanks to the activities of the startup Those Vegan Cowboys, which has a 20-strong team in a Ghent laboratory researching the production of milk protein in yeast. The company has promised to give up to 2.5 million euros to whoever finds a fungus that can convert grass into the milk protein casein. The ultimate goal is to make milk products such as cheese that taste the same as traditional varieties, but without involving cows, and therefore using less land and water, with CO_2 emissions only one fifth of those caused by cows' milk products.

Cellular farming has also already found its way into the supermarket on a modest scale. The US startup Perfect Day supplies animal-free whey protein to companies, which turn it into vegan ice cream, cream cheese and protein shakes. Whey protein is the protein with the second biggest share in milk after casein, and is a >

by-product of traditional cheesemaking. 'The ice cream tastes just like dairy ice cream, because the whey protein is exactly the same,' says Etske Bijl, from Wageningen's Food Quality and Design chair group. 'Only it's a lot easier, technically, to make whey protein for ice cream than to make casein for making cheese.'
Bijl is project leader of the 'Animal-free milk protein' consortium, which received 1.7 million euros as part of the National Science Agenda. It is a Dutch Research Council-funded project, in which a dozen research institutions and companies ¬ including Those Vegan Cowboys ¬will work together over the next four years on a great many questions surrounding casein: aspects ranging from production and purification to cheesemaking, food safety, consumer behaviour, legislation and the implications for the dairy farming sector.
Bijl: 'One of the key initial research questions is: what is the best micro-organism for producing casein, preferably in a way that means the protein ends up in the culture medium?'

EXPERIENCE WITH YEAST

The research is focusing first on a yeast species: *Pichia pastoris*. The industry already has a lot of experience with this micro-organism, which is used for making enzymes for bread and beer production. This yeast grows rapidly in heated, stainless-steel vats on a simple diet of sugar or methanol.
'We are working with researchers at Wageningen Food & Biobased Research,' says Bijl. 'They have a lot of experience with making proteins in *Pichia*. They can use it to produce large quantities of gelatine and silk protein, for example. It remains to be seen whether it will work for the milk proteins we are interested in. Casein is somewhat unusual protein because there are phosphate groups on the protein. You don't just want the yeast to make casein, but also that the phosphate groups get to where they are needed. Those phosphate groups are indispensable, because thanks to them, caseins can clump together into little balls: casein micelles. Cow's milk is full of these micelles, which play a key role in cheese production.'
'Once cow's milk starts curdling during cheese-making, the micelles cause a kind of watery gel to form. If you start with isolated casein – which can be bought as powder – no gel is formed, and there is no basis for making cheese. You can use such isolated casein to make something that resembles cheese spread or pizza cheese. But we know from consumer research that there is a demand for a substitute for a cheese like Gouda, with the same flavour and texture. If you want to achieve that, you have to start with a similar micelle structure.'

PURIFICATION

Once you can make enough casein, other important questions arise: how do you remove the protein from the culture medium, and how pure does it need to be to use it as a food ingredient? The yeast itself also excretes various substances and proteins, and the question is whether these should all be removed. In Keppler's laboratory, the culture medium will be purified step by step and analysed

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in detail. Keppler: ‘My colleagues within the consortium will then look at the protein structure, while researchers in Maastricht will test for the presence of toxic substances.’
There are other ingredients that contribute to the flavour of cheese too. Depending on the type of cheese and its age, it contains 12 to 32 per cent fat. So fat is another indispensable ingredient, for which a suitable animal-free substitute needs to be found.
During the cheesemaking process, rennet is added as well as lactic starter cultures, which are a mixture of lactic acid bacteria. According to Bijl, the flavour of cheese comes mainly from the way those bacteria break down casein, milk fat and lactose. If you replace those basic ingredients, what happens to the smell and taste of dairy-free cheese? ‘We know that if you use slightly different starter ingredients in your cheesemaking, it will directly affect the flavour of the end result. So if you use a vegetable oil as your source of fat, you can be sure the texture and flavour will be different. Cheese bacteria use lactose during the ripening process. But lactose is almost exclusively obtained from cow’s milk, precisely the source we want to avoid. So the question is whether you can use a different kind of sugar.’
This is not the only challenge, says Bijl. ‘Cheese bacteria need other milk ingredients as well to thrive, and you don’t have any of those if you start with only casein and plant-based fat. So we’re going to see whether we should add vitamins, for example, to help the bacteria grow well. Whatever you do in this project, you run up against a lot of different questions. All in all, it is very ambitious, but that was precisely one of the criteria for the Research Council funding. It won’t be easy, but potentially it could be very fruitful. If the project is successful, it will contribute to a genuinely innovative technology.’

LABELLING

The projects Keppler and Bijl are working on are not limited to questions of processing technology. Issues of legislation and labelling come in for attention as well, because getting yeast to make a milk protein requires genetic modification. If you produce food ingredients in Europe with this technology, the product has to go through an extensive registration procedure. ‘I am often asked when this kind of product will go on sale, but it partly depends on the regulations.’
Food researchers are also going to look into the digestion of the protein, and social scientists will study the wishes of consumers and the role of farmers, says Bijl. What will this technology mean for the dairy industry? ‘Ultimately, the yeasts that make the milk proteins need a source of food too, and maybe farmers can play a role there. One of the consortium partners, Those Vegan Cowboys, is looking at grass as a food source. That falls outside the scope of our project. In the lab, we grow the yeasts on methanol and sugar, but in future we do want to consider alternatives to that.’ ■

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PUBLISHING IS MORE IMPORTANT THAN PATENTING

If interesting techniques are developed in the projects working on making vegan cheese, the participating companies will get three months to apply for a patent for specific applications before the research is published. ‘Companies can’t apply for all-encompassing patents that could block these technological developments. Clear agreements have been made on that in advance, says researcher Etske Bijl.’
Ultimately, publishing is more important than registering patents, according to Bijl. Sharing insights can speed up this technological development. ‘I’m guessing that there are lots more startups working on casein around the world, but that everyone encounters the same problems. You don’t get to hear much about that because nothing is being published. For example, Perfect Day has applied for a patent for the production of casein, but nothing has been published about their method. One of the goals of our research is to be as open as we can about the bottlenecks in the microbial production of casein, to avoid everyone continually making the same mistakes.’