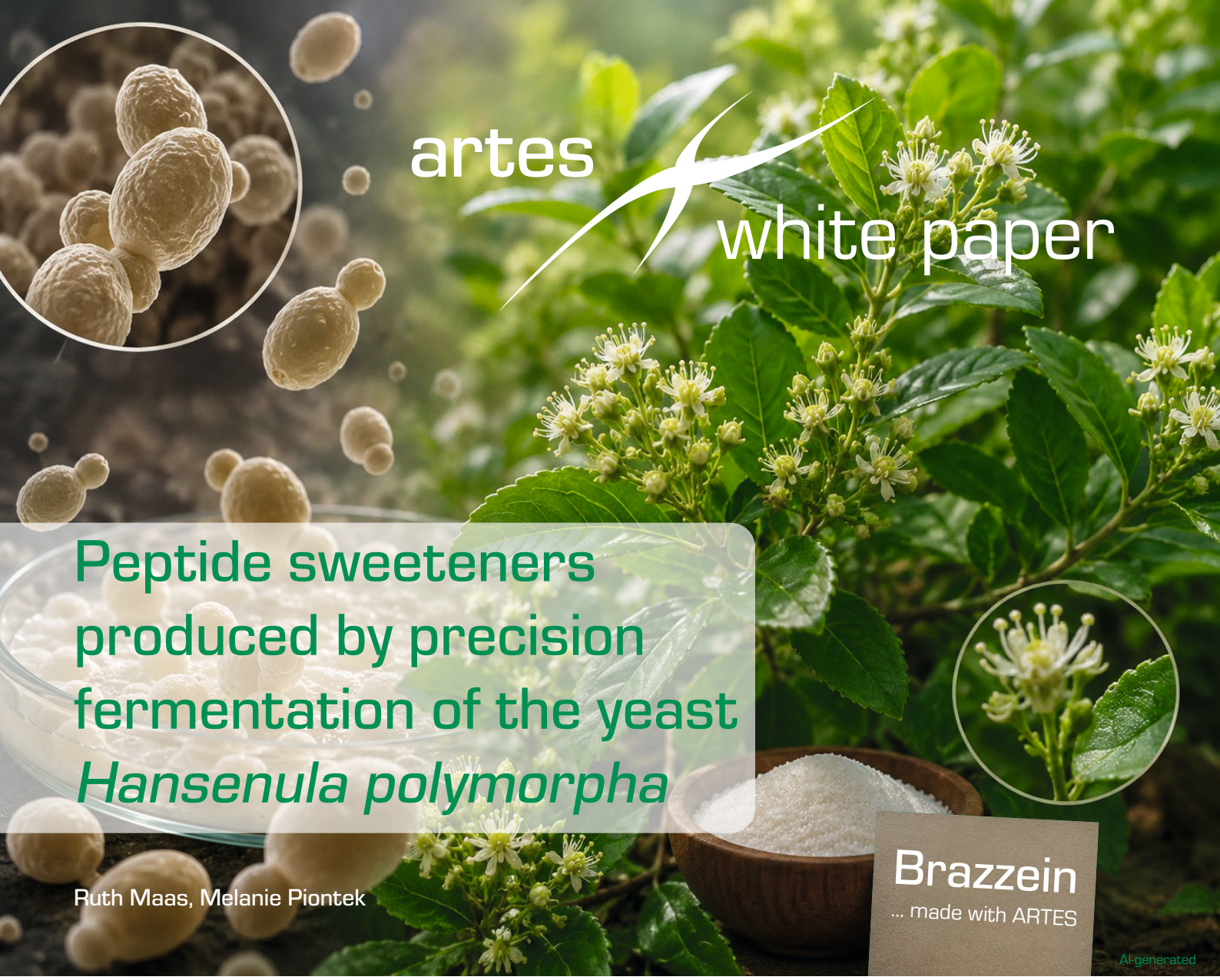

Peptide sweeteners produced by precision fermentation of the yeast *Hansenula polymorpha*

Ruth Maas, Melanie Piontek

ARTES Biotechnology uses microbial expression hosts to create engineered production cell lines and customized processes for various industry applications. The company´s primary strength lies in its extensive experience of developing robust and efficient yeast production strains based on *Hansenula polymorpha*, a yeast allowing for production free of methanol, antibiotics and antibiotic resistance genes, as well as of animal-derived components (1, 2).

One of the company's technological highlights is the production of recombinant proteins in yeast without using antibiotic resistance marker for selection or methanol as an inducer. This enables its customers to benefit from safer and more sustainable m^3-scale commercial production. Eliminating antibiotic selection avoids concerns about antibiotics and their resistance genes, which is especially important for products intended for food and feed. Methanol-free production is ideal for large-scale manufacturing processes due to the greatly reduced cost-of-goods. Product development benefits from simpler regulatory pathways, lower production costs, and easier compliance with food-grade standards. Overall, ARTES Biotechnology's development approach secures efficient production, accelerates timelines from concept to scale, and aligns with industry demands for safe, scalable solutions for food and animal feed applications. These two features of the toolbox – the development of cell lines free of antibiotic-resistance genes and of methanol-free manufacturing processes – are particularly effective in the production of peptide sweeteners such as Brazzein and Monellin.

Peptide Sweeteners as Emerging Sugar Alternatives

Peptide-based sweeteners have attracted considerable scientific and commercial interest due to their high sweetening potency and very low caloric content. Many naturally occurring sweet peptides, such as Brazzein, Thaumatin, and Monellin, originate from tropical plants, where they serve as secondary metabolites with specific biological functions. While the isolation of these products from natural sources is inefficient, production of peptide sweeteners by precision fermentation of microorganisms is an efficient and scalable approach to their industrial synthesis, offering controlled purity and consistent quality (3). Current development focuses on enhancing recombinant yields, protein stability, and downstream processing to enable the commercial use of peptide sweeteners in the food industry.

Production of Peptide Sweeteners by precision fermentation of *Hansenula polymorpha*

The production of peptide sweeteners by *H. polymorpha* without the need for antibiotics or antibiotic resistance genes eliminates the risk of spreading of antibiotic resistances via horizontal gene transfer. This process avoids residues in the final product, reduces cost-of-goods and meets strict food and feed safety standards. It also simplifies regulatory approval, enhances consumer trust, and reduces market barriers for food- and feed-related applications.

At ARTES, strain generation starts with cloning of a synthetic gene that has been optimised for the expression and secretion in *H. polymorpha*. Expression is controlled by a customized combination of strong promoters and secretion

artes white paper

leaders, selected from our toolbox to achieve superior productivity. Transformed *H. polymorpha* cells typically harbour up to 50 copies of the expression construct integrated into one of the cell's chromosomes which allows for the utilization of gene dosage effects to generate and select top producers as production strains.

Brazzein

Brazzein is a highly water-soluble, heat-stable protein with an intensely sweet taste that resembles that of carbohydrate sweeteners. It was isolated in two forms from the fruit of the West African climbing plant *Pentadiplandra brazzeana Baillon*. Along with Pentadin, discovered in 1989, Brazzein is the second sweet protein that was discovered in this fruit. The major form contains a pyroglutamate at its N-terminus, while the minor form lacks this N-terminal pyroglutamate. The major form is 750-times sweeter than sucrose on a weight basis (4).

In methanol-free precision fermentations of engineered *H. polymorpha* strains, high productivities of several grams secreted product per litre in synthetic media was achieved for native Brazzein. Minimal host-, product- and process-related impurities such as colourants and residuals from complex media simplified the purification of high quality Brazzein bulk product. A simple, one-step purification process is sufficient to achieve a purity of over 99%. ARTES´ process replaces classical, expensive chromatography operation by an 8 – 10-fold less expensive alternative.

For both, Brazzein and Brazzein variants with even sweeter taste and/or faster taste onset technology is now available for robust, elegant and inexpensive production.

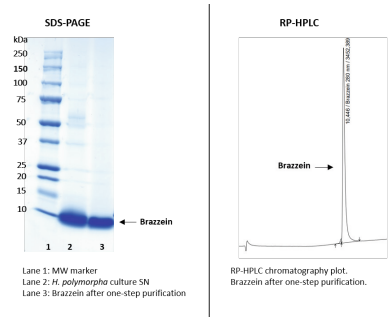

Figure 1: SDS Gel (left), RP-HPLC chromatogram (right)

Monellin

Monellin is a naturally occurring sweet protein that was first discovered in the 1960s in the fruit of the West African plant *Dioscoreophyllum cumminsii*, commonly known as the serendipity berry. Monellin is extremely sweet, being 4000-times sweeter than sucrose on a weight basis, while containing only very few calories. It is a two-chain protein composed of non-covalently linked A- and B-chains (4). Both chains are required to form the correct three-dimensional structure responsible for its intense sweetness. For recombinant production, Monellin can be expressed as a single chain with a short peptide linker between the A- and B-chains.

High productivity was achieved in precision fermentation of an engineered Monellin-secreting *H. polymorpha* strain. Cell-free culture supernatants of the mitotically stable *H. polymorpha* strain contained Monellin at a concentration of several g/L with very few impurities. As previously demonstrated for Brazzein, this enables a simple, fully scalable purification process for Monellin.

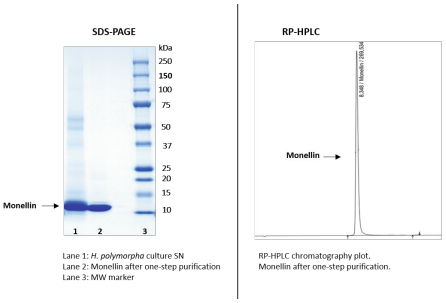

Figure 2: SDS-Gel (left), RP-HPLC-Chromatogram (right)

Summary and outlook

The secretory production of the peptide sweeteners Brazzein and Monellin was successfully established at ARTES. The recombinant production strains have been proven to be genetically stable for hundred generations of growth under non-selective conditions, ensuring reliable productivity at all production scales. Brazzein and Monellin were both found in the supernatant of *H. polymorpha* cultures at a concentration of close to 5 g/L with very few impurities, enabling simple purification processes.

Processes for these peptide sweeteners, as well as for several other commercially attractive products are "ready-to-transfer" at ARTES and are available for licensing on short term.

For more information, please visit our website!

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Made with ARTES:

ARTES Biotechnology offers its customers the development of microbial cell lines and production processes for the manufacture of their products. We add years of experience in recombinant protein manufacturing to your project. Please contact us for more information.

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