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The sweet protein brazzein as a promising natural sweetener

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In the modern world, due to the overconsumption of sugar-containing products, the problem of obesity is relevant. Among the many sweeteners that minimize sugar intake, a group of sweet-tasting proteins is up-and-coming. Brazzein is the smallest of the sweet proteins (54 aa, 6473 Da), and it is also safe for obese and diabetic people since it does not affect blood sugar and insulin levels. Brazzein has high thermal stability over a wide pH range: from 2 to 8 [1]. To increase the level of sweetness of brazzein, mutant variants of this protein were created through site-directed mutagenesis, the sweetest of which is triple mutant H31R/E36D/E41A, which is 22,500 times sweeter than sucrose [2]. Since the content of brazzein in the fruits of the natural source (*Pentadiplandra brazzeana*) is extremely low (0.2%), various methods have been developed to obtain brazzein using heterologous expression systems, which used as producers: bacteria (*Escherichia coli*, *Lactococcus lactis*), yeast (*Pichia pastoris*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*), plants (*Zea mays*, *Oryza sativa*, *Lactuca sativa*, *Nicotiana tabacum*) and animals (*Mus musculus*) [3–5]. Despite the short peptide sequence, the industrial production of recombinant protein faced several problems, including low protein yield (e.g in mouse milk it was detectable on western blot analysis only) and loss of sweetness. An extremely relevant and promising way to obtain recombinant brazzein is the optimization of extracellular expression in bakers yeasts with the GRAS (Generally recognized as safe) status, since the safety of these microorganisms for human health can potentially significantly reduce the number of brazzein purification steps and thereby reduce its cost to consumers.

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