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Critical variants increasing the sweetness of sweet-tasting protein, brazzein

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The demand for non-calorigenic protein-based sweeteners with favorable taste properties is high. Brazzein, a sweet-tasting protein with a molecular mass of 6.4 kDa, was originally isolated from the fruit of the West African plant *Pentadiplandra brazzeana* Baillon. Brazzein is the smallest naturally occurring sweet-tasting protein described to date and provides an optimal system for investigating the chemical and structural requirements of the sweet-taste response. Particularly, brazzein is an ideal candidate for a low-calorie sweetener because of its natural origin and favorable physical properties. Brazzein is also the most heat-stable and pH stable member of the set of proteins known to have intrinsic sweetness. Brazzein is approximately 2,000 times sweeter than sucrose. To develop highly sweet protein, in this study, we constructed several brazzein variants by site-directed mutagenesis. The brazzein variants were expressed in *E. coli* BL21 and yeast *Kluyveromyces lactis* which is recognized as GRAS. Some of brazzein variants were not sweeter than the wild-type brazzein. The variants of the residues that located in the loop between β-strand III and β-strand III showed similar sweetness to the wild-type brazzein. On the other hand, the variants of the residues that located in the β-strand III in loop between α-helix and β-strand and the residues in N- and C- termini increased sweetness. Moreover, the introduction of multiple variants results in stronger sweetness, showing approximately 5,000-22,500 times sweeter than sucrose (g/g).The increasing order of their sweetness were triple variants>double variants>single variants. This study offers information on the precise interaction mechanism of brazzein with human sweet taste receptor responsible for the sweetness of brazzein and will be of great value in future design of brazzein sweeter variants.

Biography

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