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Brazzein a Small, Sweet Protein: Discovery and Physiological Overview

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Introduction

For many years, only small molecules were considered capable of inducing a sweet taste (cf. Inglett, 1974). This idea was shattered when miraculin from the miracle fruit of *Synsepalum dulcificum* was shown to have (Brouwer *et al.*, 1968; Kurihara and Beidler, 1968) a mol. wt of 24 600.

The search for sweeteners was no longer limited to small molecules and resulted within a few years in the discovery of monellin and thaumatin (Morris and Cagan, 1972; van der Wel and Loeve, 1972). Later, mabinlin and curculin were discovered (Hu and He, 1983; Yamashita *et al.*, 1995).

In the 1980s, our attention was attracted to a West African plant, *Pentadiplandra brazzeana* (Hladik *et al.*, 1984). We obtained a small sample of smoke-dried berries in which we tentatively identified a sweet tasting protein, pentadin (van der Wel *et al.*, 1989).

From a new and fresher sample of the berries' pulp we identified and isolated the major sweet principle of *P. brazzeana*, which we named brazzein (Ming and Hellekant, 1994). Brazzein is distributed in the pulp between the epicarp the seeds and turns from green to red during ripening (Figure 1a). The content of brazzein in the ripe fruit appears to be roughly 0.2–0.05% by weight.

General properties

Brazzein is the smallest (mol. wt 6473) and one of the sweetest of the protein sweeteners discovered so far. It is composed of a single chain of 54 amino acid residues: pyrE-D-K-C-K-K-V-Y-E-N-Y-P-V-S-K-C-Q-L-A-N-Q-C-N-Y-D-C-K-L-D-K-H-A-R-S-G-E-C-F-Y-D-E-K-R-N-L-Q-C-I-C-D-Y-C-E-Y.

The major form of brazzein isolated from its natural source contains (~80%) pyrE at its N-terminus. The remainder (~20%) is des-pyrE. Sensory analyses shows that the pyrE containing brazzein is 500 times sweeter than a 10% sucrose solution on a weight basis. Removal of the pyrE increases its sweetness with a factor of two (Izawa *et al.*, 1996). The water solubility of brazzein is at least 50 mg/ml, i.e. >7.7 mM.

Brazzein is exceptionally heat stable and its sweet taste remains after incubation at 98°C for 2 h and at 80°C for 4.5 h in the pH range of 2.5–8 (Ming and Hellekant, 1994). This may be the result of its four intramolecular disulfide bonds and lack of free sulfhydryl groups, because nuclear magnetic resonance (NMR) studies show that these bridges are evenly distributed in brazzein (Caldwell *et al.*, 1998a,b).

Physiological properties

Indigenous people have known brazzein for centuries. It is consumed either raw or in a cooked form (Hladik and Hladik, 1988) and used as a sweetening agent in drinks and food.

We have studied the taste of brazzein in humans and animals. We recorded from the chorda tympani taste nerve (CT) in non-human primates (Hellekant *et al.*, 1997a,b; Danilova *et al.*, 2002). We found that brazzein in Old World primates stimulated nerve fibers that respond to sweeteners (S fibers) and was preferred. In New World monkeys it gave no response.

Recently we extended the structure-function studies in rhesus monkeys and humans (Jin *et al.*, 2003a,b). As stimuli we used des-pGlu brazzein, 25 brazzein mutants and monellin. All proteins were dissolved to a concentration of 100 µg/ml and adjusted to pH 7.0.

In the monkey study we recorded from single S taste fibers the responses to taste stimulation with the above compounds. The S fibers were selected based on their sensitivity to sweet stimuli.

In the human study the subjects scored the sweetness of taste stimuli with a semantically labeled scale for rating sensation intensity. The human results were then combined with the electrophysiological from the monkeys.

Both methods showed that different mutations at position 29 (changing Asp29 to Ala, Lys or Asn) made the molecule significantly sweeter than brazzein, while mutations at positions 30 or 33 (Lys30Asp or Arg33Ala) removed all sweetness (Figure 1b). The same pattern occurred again at the β-turn region, where Glu41Lys gave the highest sweetness score, whereas a mutation two residues distant (Arg43Ala) abolished the sweetness. These findings indicate that charge is important for eliciting sweetness, whereas the length of the side-chain plays a lesser role. We also found that the N- and C-termini are important for the sweetness of brazzein.

Figure 1a

Figure 1b

Figure 1 **(a)** Photo of *Pentadiplandra brazzeana* fruit. **(b)** Backbone ribbon diagram of wild type brazzein showing positions of mutations and the corresponding changes in taste (red, increased sweetness; black, the same; light blue, decreased sweetness in comparison with WT brazzein; dark blue, scored as water).

Another major finding was the close correlation ($r = 0.78$) between the results in humans and monkeys. This supports strongly our hypothesis that S fibers from the rhesus monkey recordings can be used to assess sweetness in humans (cf. Hellekant *et al.*, 1998).

Applications

Sweet carbohydrates have several problems associated with their use, such as high caloric content, tooth decay and diabetes mellitus. This leads to demand for non-sugar alternatives. However, non-sugar alternatives show also deficiencies such as being unsuitable in most cooking or baking applications. Their organoleptic qualities are also inferior and concern from a toxicological point of view can be raised for sweeteners, such as synthetic sweeteners, which lack a history of human consumption.

Brazzein combines a long history of human consumption, small size with high sweet potency, solubility and exceptional thermostability. It tastes purely sweet with no sourness, saltiness or bitterness. These qualities make it a very good alternative. However, as is a characteristic of many high intensity sweeteners, the sweetness of brazzein grows slightly slower than that of sucrose. The sweetness of brazzein is readily washed from the tongue and neither mouth cooling nor extensive lingering occur. It often improves the mouth feel of beverages when blended with other sweeteners and works well in both citric acid and phosphate beverage systems.

Brazzein combines well with most high intensity sweeteners such as acesulfame-K and aspartame, providing both quantitative and qualitative synergy. Also it improves stability, flavor and mouth feel when blended with acesulfame-K and aspartame, either alone or blended. It typically reduces the side taste of other sweeteners; for example, a blend of stevioside and brazzein is superior in taste quality to stevioside alone. Brazzein has been expressed in yeast (Guan *et al.*, 1995), fruits and vegetables to increase their sweetness and in grains to be economically extracted and used as a sweetened flour (cf. Faus, 2000).

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