Brazzein
Sweetener with a future?

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1. General Information

Brazzein is a sweet protein isolated from the fruit of the West African climbing plant *Pentadiplandra Brazzeana*. It has compared to sugar a 500 to 2,000 times sweeter taste. Furthermore, it has low calorie content and high temperature resistance. This is a good combination to use it in food industry as a sugar substitute.

Brazzein was first discovered by two researchers in Africa, who observed by studying the behavior of monkeys that the animals largely subsisted on the red fruits of *Penta brazzeana*. They examined the plant and discovered its intense sweetness. In 1994 Brazzein was first isolated from the fruit at the University of Wisconsin-Madison.

Brazzein is claimed by the University of Wisconsin-Madison as a separate invention, although the fruit of the plant has been known for a long time in Africa. Overall, the University holds three patents on compounds isolated from the plant and on the industrial production of Brazzein.

2. Structure

**Tertiary Structure:**
Brazzein is a sweet protein consisting of 54 amino acids, which can be found either in its crystalline state or in its solvated state, which is slightly different. The tertiary structure contains in the crystalline state 2 α-helices and three antiparallel β-sheets. In the solvated state the molecule lacks one α-helix. Also the loops between the helices and β-sheets are not to be neglected since they play an important role for the sweetness of the molecule and for that reason are referred to as “sweet fingers”.

Furthermore the crystalline structure is formed by two Brazzein molecules; therefore it becomes a homodimer, which is stabilized by six H-bonds. Meanwhile the solvated structure is a monomer.
Amino acids sequence (wild type):

There is more than one possible sequence, for example the first acid, the Glu1, has been exchanged by pyroglutamat which enhances the sweetness by the factor two. There are also variations where the first amino acid is completely missing, resulting in a Brazzein molecule consisting of only 53 amino acids.

Details:
The molecule has four disulfide bonds, which stabilize the conformation. The bonds are between Cys4-Cys52, Cys16- Cys37, Cys22- Cys47, Cys26- Cys49.
The α-helix which only forms in the crystalline form is called α-1-helix and contains the amino acids 13-17, the other helix is slightly larger and contains the amino acids 20-31. The three antiparallel β-sheets are formed by the amino acids 5-7, 34-39 and 45-50. The loops in between, especially the 9-19-loop, are important for the sweetness.

In the crystalline structure the homodimer is connected through the 6 H-Bonds between Gln17O---Ala19’N, Asn20N---Cys37O, Cys37O---Asn20’N, Tyr2O---Glu36O, Ala19N---Glu17’O, Glu36O---Tyr24’O

Sweetness:
The result of mutating different amino acids was, that there are 3 different groups of amino acids in the molecule, which are sorted by the impact they have on the sweet taste of Brazzein.
The amino acids are divided into the critical, the important and the involved ones as following:

Five (5) amino acids were found to be critical: Lys30, Arg33, Glu36, Tyr39, Arg43;
seven (7) amino acids were found to be important: Lys5, Lys6, Tyr8, Lys15, His31, Lys42, Asp50;
and four (4) amino acids were found to be involved: Gln17, Asp29, Asp40, Glu41.

Another interesting fact is that from the critical ones, Lys30 and Glu36 are on the opposite site of the molecule, which implies that the reaction with the human sweetness receptor is taking place on multiple sites.

Scientists have also defined three different sites, which are operatively distinguished. In that manner site 1 is defined as the loop 43 which contains the amino acids: Tyr39, Asp40, Glu41, Lys42, and Arg43. The loop 43 is a type-1-β-turn. As you can see the site 1 contains two critical amino acids, in particular Arg43 and Tyr39 which are forming a cation-π-interaction. The side chain of Arg43 is also interacting with the residue Glu41 electrostatically and therefore stabilizes the conformation.

Site 2 is basically the termini of the protein as well as some residues of the central part, which are keeping the conformation in shape. For example, Lys30 and Tyr8 have a cation-π-bond which stabilizes the α-2-Helix, as well as the loop between β1 and α1. The interaction of the side chains of Arg33 and Tyr54 stabilizes the arrangement of the β-1-sheet and the C-terminal part. The relative position of β1 and β3 is determined by the electrostatic interaction of Lys27 with Asp30 and Glu36. Glu36 and Lys6 seem to be fully exposed to the solvent which leads to the conclusion that they interact with the sweetness receptor, as well as with the N-terminal parts. Site 3 is referred to as loop 9-19. It is located at the α-2-helix and besides a few residues, which are keeping the whole loop in the right shape. It is known, that Glu17 is significant for the sweet taste. Firstly, it is an exposed residue, so it can react very well with the sweetness receptor and secondly, the mutation of the Glu17 lowers the sweetness significantly.

3. Synthesis

3.1 Synthesis with E.coli

The extraction of the protein brazzein from its natural source, which is the West African plant Pentadiplandra Brazzeana, is very expensive and therefore not profitable. To find an alternative option for cheaper mass production people try to use recombinant DNA technology.
They designed a brazzein gene on the basis of the amino acid sequence, synthesized by using two oligonucleotides which were ligated together as one:

F1; 5’ATGGACAAATGTAAGAGTATACGAAACTACCCGGTATCCAAATGTCAGCTGGCAGAGCAG TGTAACTACGACTGAAACACGCTCG-3’
and F2; 3’TGCTGACATTTGACCTGTTTGTGCGAGCAAGGCCACTTACGAAGATGCTGCTTTTTGCAT TGGACGTCAGTAGACGCTGATGACGCTTATTGATT-5’.

Additional a starting codon (Met) was added to the first sequence. The nucleotide sequence was divided into two partially overlapping oligonucleotides, which were annealed at 65°C to create DNA double strands. This fragment was amplified by PCR using a special DNA polymerase in a thermal cycler, which regulates the temperature during the reaction. The correct DNA sequence was confirmed by automated DNA sequencing and then it was digested with BamH1 enzyme and extracted from gel. The purified DNA-fragment was inserted into a special vector with a strong promoter and then transformed in E. coli bacteria (Top10F). Bacterial colonies were screened by agar plates containing X-gal (Roche, Mannheim, Germany) to discriminate between recombinant and non-recombinant bacteria by making use of the blue-white screen. Colonies were mass cultured in LB medium supplement with a low dose of ampicillin in an incubator at 37°C. Then recombinant plasmids were identified and selected by using PCR and automated DNA sequencing. The expression vectors were transformed into another special E. coli strain (BL21DE3plysS), which has been suggested to give rise to increased expression levels of secreted protein by reduction of proteolysis proteins. The recombinant protein is expressed as a soluble form after induction by IPTG which is a mimic of allo lactose, a synthetic Inductor of the lac-Operon, which regulates gene expression in E. coli. Thus protein production starts with the binding of IPTG to the lac-repressor. Finally the cells were collected by centrifugation and the protein was detected by SDS-PAGE.

### 3.2 with Maize

Brazzein can also be synthesized in genetically engineered maize. This method will probably be used to produce Brazzein for commercial industry if it gets the permit for the use in food. Therefore the Brazzein gene was engineered into the maize DNA and was connected with an embryo-preferred promoter and targeted to the cell wall.
The accumulation of the protein was tested with ELISA (enzymelinked immunosorbent assay) and so it turned out that the highest brazzein concentration was in the germ.

To purify the sweet protein the isolated germ runs through different fractions and as result out of one ton of corn one kilogram brazzein can be synthesized nowadays. That equates in sweetness about 1000 kilograms of common sugar.

Brazzein can be enriched up to 70% of soluble protein by this method but to use it in food industries the Brazzein containing germ can be left as it is and germ flour can be produced. This makes the commercial use much easier because the flour is already sweet and the fractioning process can be left out. The use of Brazzein flour was already tested in Protein Bars and Muffins by ProdiGene, who also have developed this way of synthesis, and the results were very positive: “exhibited a smoother, more sugar-like taste profile”, “slightly warmer and fuller” mouthfeel than the bar containing control germ.

### 6. Use

Brazzein can be used as a sweetener for the food industry because of its long-lasting and strong sweetness. It can be 500 to 2000 times sweeter than sucrose. Moreover, it has low calorie content and it is acceptable for diabetics. Brazzein is water soluble (>50mg/mL) and is stable over a broad pH range from 2.5 to 8. In comparison to other sweeteners Brazzein is more heat stable and survives 2 hours at 98°C without losing its sweetness. Due to its high heat stability it can be used for pastries without losing their sweetness during baking, while other sweetener proteins would denature. This fact makes the protein very interesting for the food industry.
Another reason for using Brazzein as a sweetener is that Brazzein tastes more like actual sugar than other sweeteners. When this protein is blended with other sweeteners, such as Aspartame and Stevia, Brazzein complements their flavor.

So far, no side effects were detected at the African people who consume the fruits that contain Brazzein but right now Brazzein is not yet available on the free market.

Brazzein is also used for studies about the sweet taste receptor. The German Institute of Human Nutrition in Potsdam makes use of Brazzein and the protein Gurmarin, which blocks the sweet taste of rodents, in their research. The scientists search for the interior design of the sweet taste receptor. Therefore, they use specific mutagenesis on Brazzein and Gurmarin but also on the sweet taste receptor of humans and rodents.

5. Sources

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