Claims

1. A method for preparing Brazzein or a mutant thereof, comprising:

   1) constructing an expression vector, the expression vector comprising a coding sequence of the Brazzein or the mutant thereof and a coding sequence of a leader peptide, wherein the leader peptide is derived from *Rhizopus oryzae* lipase; and

   2) introducing the expression vector into a host cell.

2. The method of claim 1, wherein the Brazzein comprises an amino acid sequence as set forth in SEQ ID NO: 1; and the mutant comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 1 and has a sweet taste.

3. The method of claim 1 or 2, wherein the Brazzein or the mutant thereof has a sweetness threshold below 100 μg/mL, preferably below 10 μg/mL, more preferably below 1 μg/mL.

4. The method of any one of claims 1-3, wherein the mutant comprises amino acid substitutions E35D, E40A, E40D, E40K, E40R, H30R, or any combination thereof; preferably, the mutant comprises any of the following combinations of amino acid substitutions:

   1) E35D/E40A;

   2) E35D/E40D;

   3) E35D/E40K;

   4) E35D/E40R;

   5) H30R/E35D/E40A;

   6) H30R/E35D/E40K; and

   7) H30R/E35D/E40R.

5. The method of any one of claims 1-4, wherein the mutant comprises an amino acid sequence as set forth in any one of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15.

6. The method of any one of claims 1-5, wherein the leader peptide comprises an amino acid sequence as set forth in SEQ ID NO: 17 or an amino acid sequence having at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 17.

7. The method of any one of claims 1-6, wherein the Brazzein or the mutant thereof and the leader peptide are expressed in the form of a fusion protein; preferably, the leader peptide is located at the N-terminus of the fusion protein.

8. The method of any one of claims 1-7, wherein the expression vector further comprises any one or more selected from the group consisting of:

(i) a coding sequence of a short peptide providing a protease cleavage site, wherein the coding sequence of the short peptide is located between the coding sequence of the leader peptide and the coding sequence of the Brazzein or the mutant thereof; preferably, the short peptide comprises amino acid sequence VALEKR;

(ii) a coding sequence of a signal peptide; preferably, the signal peptide is derived from *Aspergillus oryzae* α-amylase;

(iii) promoter and terminator sequences; preferably, the promoter and terminator are derived from an *Aspergillus oryzae* α-amylase gene; and

(iv) a selectable gene, preferably an *amdS* gene.

9. The method of any one of claims 1-8, wherein the host cell is a filamentous fungus, preferably *Aspergillus oryzae*, more preferably *Aspergillus oryzae* NBRC4177.

10. Brazzein or a mutant thereof prepared by the method of any one of claims 1-9.

11. A nucleic acid molecule comprising a coding sequence of the Brazzein or the mutant thereof of claim 10.

12. An expression vector comprising a coding sequence of the Brazzein or the mutant thereof prepared by the method of any one of claims 1-9 and a coding sequence of a leader peptide, wherein the leader peptide is derived from *Rhizopus oryzae* lipase.

13. The expression vector of claim 12, further comprising any one or more selected from the group consisting of:

(i) a coding sequence of a short peptide providing a protease cleavage site, wherein the coding sequence of the short peptide is located in the 3' direction of the coding sequence of the leader peptide and in the 5' direction of the coding sequence of the Brazzein or the mutant thereof;

(ii) a coding sequence of a signal peptide; preferably, the signal peptide is derived from *Aspergillus oryzae* α-amylase;

(iii) promoter and terminator sequences; preferably, the promoter and terminator are derived from an *Aspergillus oryzae* α-amylase gene; and

(iv) a selectable gene, preferably an *amdS* gene.

14. The expression vector of claim 12 or 13, comprising a nucleotide sequence as set forth in any one of SEQ ID NOs: 20, 22, 24, 26, 28, 30, 32 and 34, or a nucleotide sequence having at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to the nucleotide sequence as set forth in any one of SEQ ID NOs: 20, 22, 24, 26, 28, 30, 32 and 34.

15. An edible product comprising the Brazzein or the mutant thereof of claim 10, preferably, the edible product is a food, a beverage or a medicament; more preferably, the edible product further comprises a sweetener different from the Brazzein or the mutant thereof; even preferably, the sweetener includes sucrose; more preferably, the beverage is a yogurt beverage or a sparkling water, such as a sugar-free sparkling water.