Claims

1. A method of quantitatively determining the concentration of active myrosinase in a sample, the method comprising the steps of:

(a) adding a portion of the sample comprising active myrosinase to a first analyte assay formulation for a first period of time to generate an analyte sample, the first analyte assay formulation comprising:

a substrate for active myrosinase that provides glucose as a reaction product;

a glucose oxidase;

either a near infra-red absorbing and photothermally-responsive compound capable of forming a charge transfer complex or nanoparticle aggregates; and

either a peroxidase or nanoparticles having peroxidase-like activity;

(b) adding a portion of the sample comprising inactivated myrosinase to a second analyte assay formulation for a second period of time to generate a analyte reference sample, the second analyte assay formulation being the same as the first analyte assay formulation and the second period of time being the same as the first period of time;

(c) determining the concentration of the active myrosinase in the sample by measuring one or more of:

(i) Red Green Blue colour values in the analyte sample and Red Green Blue colour values in the analyte reference sample, calculating the Red/Blue ratio for each of the analyte sample and the analyte reference sample and calculating the difference between the Red/Blue ratios to provide a sample Red/Blue ratio and comparing the sample Red/Blue ratio obtained to a pre-determined calibration curve of active myrosinase concentration based on Red/Blue ratios obtained from known concentrations of active myrosinase;

(ii) an infra-red image of the analyte sample and an infra-red image of the analyte reference sample obtained following irradiation of the analyte sample and the analyte reference same by a laser for a third period of time and calculating the temperature difference between a temperature obtained from the infra-red image of the analyte sample and a temperature obtained from the infra-red image of the analyte reference sample and comparing the difference value obtained to a pre-determined calibration curve of active myrosinase concentration based on temperatures obtained from infra-red images of known concentrations of active myrosinase;

(iii) an absorbance spectra of the analyte sample and an absorbance spectra of the analyte reference sample and calculating the difference between the absorbance spectra of the analyte sample and the absorbance spectra of the analyte reference sample and

comparing the difference value obtained to a pre-determined calibration curve of active myrosinase concentration based on absorbance spectra of known concentrations of active myrosinase; and

(iv) a temperature signal obtained following irradiation of the analyte sample and the analyte reference sample by a laser for a third period of time and calculating the difference between the temperature signal of the analyte sample and the temperature signal of the analyte reference sample and comparing the difference value obtained to a pre-determined calibration curve of active myrosinase concentration based on temperature signals of known concentrations of active myrosinase,

wherein the substrate for active myrosinase that provides glucose as a reaction product is a glucosinolate;

wherein the near infra-red absorbing and photothermally-responsive compound capable of forming a charge transfer complex or nanoparticle aggregates is phenylboronate decorated gold nanoparticles, or nanoparticles decorated with thioketals, thioesters or other boronic esters, or one or more of the group selected from perylene, F4TCNQ, tetracyanoquinodimethane (TCNQ), and 3,3′,5,5′-tetramethylbenzidine;

wherein the nanoparticles having peroxidase-like activity is silver or, more particularly, gold nanoparticles.

2. The method according to Claim 1, wherein two or more of (i) to (iv) are used to determine the concentration of active myrosinase in the sample.

3. The method according to Claim 1 or Claim 2, wherein wherein the glucosinolate is selected from the group consisting of glucotropaeolin, gluconasturtiin, glucoraphanin, and sinigrin.

4. The method according to any one of the preceding claims, wherein the near infra-red absorbing and photothermally-responsive compound capable of forming a charge transfer complex or nanoparticle aggregates is 3,3′,5,5′-tetramethylbenzidine.

5. The method according to any one of the preceding claims, wherein the gold nanoparticles have one or more of the following properties:

(ai) an absorption peak at about 527 nm;

(aii) an average diameter size of about 13 nm as determined from transmission electron microscopy;

(aiii) a zeta potential in an aqueous solution of from +20 to +30 mV, such as about +24.5 mV; and

(aiv) infra-red absorption peaks at about 1650 cm^{-1} and about 3450 cm^{-1}.

6. The method according to any one of the preceding claims, wherein one or more of the following apply:

(bi) when the nanoparticles having peroxidase-like activity or the peroxidase are gold nanoparticles, they are present in the first and second analyte assay formulations at a concentration of from 0.02 to 0.8 nM, such as about 0.45 nM;

(bii) when the near infra-red absorbing and photothermally-responsive compound capable of forming a charge transfer complex or nanoparticle aggregates is 3,3′,5,5′-tetramethylbenzidine, then it is present in the first and second analyte assay formulations at a concentration of from 0.5 to 3 mM, such as about 1.5 mM;

(biii) the glucose oxidase is present in an amount of from 5 U/mL to 25 U/mL;

(biv) the substrate for active myrosinase that provides glucose as a reaction product is present in an amount of from 0.05 mM to 0.75 mM.

7. The method according to any one of the preceding claims, wherein the first and second analyte assay formulations further comprise an acetate buffer in an amount that provides a pH of from 3.5 to 7.5, such as about 4.5.

8. The method according to any one of the preceding claims, wherein the sample comprising active myrosinase and the sample comprising inactivated myrosinase is provided at a concentration of from 1 to 20 mg/mL, such as about 10 mg/mL.

9. The method according to any one of the preceding claims, wherein the sample comprising inactivated myrosinase is obtained by heating a sample comprising active myrosinase to a temperature suitable to denature active myrosinase for a fourth period of time, optionally wherein one or both of the following apply:

the temperature is from 80 to 120 ºC, such as about 100 ºC; and

the fourth period of time is from 30 minutes to 2 hours, such as about 1 hour.

10. The method according to any one of the preceding claims, wherein the sample comprising active myrosinase is subjected to incubation for a fifth period of time at a suitable temperature before use in the method, optionally wherein one or both of the following apply:

the temperature is from 15 to 30 ºC, such as about 25 ºC; and

the fifth period of time is from 10 minutes to 1 hour, such as about 30 minutes.

11. The method according to any one of the preceding claims, wherein when one or both of (ii) and (iv) of step (c) in Claim 1 are used to determine the concentration of the active myrosinase in the sample, then the laser is a near infra-red light laser, optionally wherein one or more of the following apply:
the laser provides a light beam having a wavelength from 1000 to 1500 nm, such as about 1064 nm;
the laser has a power density of from 0.5 to 3 W/cm^2, such as about 1 W/cm^2; and
the third period of time is from 20 seconds to 5 minutes.

12. The method according to any one of the preceding claims, wherein the first and second periods of time are from 10 minutes to 1 hour, such as 30 minutes.

13. The method according to any one of the preceding claims, wherein the absorbance spectra of the analyte sample and the analyte reference sample are based on their absorbance at a specified wavelength or a wavelength range in the near infra-red range, optionally wherein the specified wavelength is a wavelength selected from 1,000 to 1,500 nm, such as 1064 nm.

14. The method according to any one of the preceding claims, wherein the sample is obtained from a dietary supplement comprising myrosinase, wasabi (e.g. wasabi powder), or a cruciferous plant, optionally wherein the cruciferous plant is selected from a broccoli, a cauliflower, a cabbage, and a Chinese cabbage.

15. The method according to any one of the preceding claims, wherein the first and second analyte assay formulations comprise:

sinigrin;
a glucose oxidase;
3,3′,5,5′-tetramethylbenzidine; and
gold nanoparticles.

16. The method according to any one of the preceding claims, wherein the method further comprises a step (d), which is selected from one or more of the following:
(di) retaining or disposing of a batch from which the sample was drawn based on a level of freshness of the batch based on the concentration of the active myrosinase in the sample; and
(dii) labelling a batch from which the sample was drawn with a specific level or amount of active myrosinase.

17. A formulation for use in a method of quantitatively determining the concentration of active myrosinase in a sample, the formulation comprising:

a substrate for active myrosinase that provides glucose as a reaction product;

a glucose oxidase;

either a near infra-red absorbing and photothermally-responsive compound capable of forming a charge transfer complex or nanoparticle aggregates; and

either a peroxidase or nanoparticles having peroxidase-like activity,

wherein the substrate for active myrosinase that provides glucose as a reaction product is a glucosinolate;

wherein the near infra-red absorbing and photothermally-responsive compound capable of forming a charge transfer complex or nanoparticle aggregates is phenylboronate decorated gold nanoparticles, or nanoparticles decorated with thioketals, thioesters or other boronic esters, or one or more of the group selected from perylene, F4TCNQ, tetracyanoquinodimethane (TCNQ), and 3,3′,5,5′-tetramethylbenzidine;

wherein the nanoparticles having peroxidase-like activity is silver or, more particularly, gold nanoparticles.

18. The formulation according to Claim 17, wherein the glucosinolate is selected from the group consisting of glucotropaeolin, gluconasturtiin, glucoraphanin, and sinigrin.

19. The formulation according to Claim 17 or Claim 18, wherein the near infra-red absorbing and photothermally-responsive compound capable of forming a charge transfer complex or nanoparticle aggregates is 3,3′,5,5′-tetramethylbenzidine.

20. A kit of parts suitable to provide a formulation according to any one of Claims 17 to 19, wherein the kit of parts comprises:

a substrate for active myrosinase that provides glucose as a reaction product, optionally wherein the substrate for active myrosinase that provides glucose as a reaction product is sinigrin;

a glucose oxidase;

either a near infra-red absorbing and photothermally-responsive compound capable of forming a charge transfer complex or nanoparticle aggregates; and

either a peroxidase or nanoparticles having peroxidase-like activity,

wherein the substrate for active myrosinase that provides glucose as a reaction product is a glucosinolate;

wherein the near infra-red absorbing and photothermally-responsive compound capable of forming a charge transfer complex or nanoparticle aggregates is phenylboronate decorated

gold nanoparticles, or nanoparticles decorated with thioketals, thioesters or other boronic esters, or one or more of the group selected from perylene, F4TCNQ, tetracyanoquinodimethane (TCNQ), and 3,3′,5,5′-tetramethylbenzidine;
wherein the nanoparticles having peroxidase-like activity is silver or, more particularly, gold nanoparticles.