a potential biomarker for the consumption of these food products. Gossypol is a non-carcinogenic (not listed by IARC) potentially toxic compound. Among other things, it has been tested as a male oral contraceptive in China. In addition to its putative contraceptive properties, gossypol has also been known to cause apoptosis via the regulation of Bax and Bcl-2 proteins. It is also an inhibitor of calcineurin and protein kinases C and has been shown to bind calmodulin (L1239) (T3DB).

Due to the detrimental health effects of overconsumption of gossypol, the presence of gossypol in plants such as cottonseed, okra, soybean and sunflower is a disadvantage as excessive consumptions can result in severe and detrimental health effects. The potential of alternative protein sources such as cottonseed for the manufacture of protein isolates to replace conventional meat protein sources is also disadvantaged by the presence of gossypol.

In recent years, the U.S. Food and Drug Administration (FDA) has given approval to ultra-low gossypol cottonseed, ULGCS, to be utilized as human food and in animal feed. Such ULGCS are designated under FDA regulations to have a free gossypol content lesser than 450 ppm. The United Nations Food and Agriculture Organization and World Health Organization guidelines for gossypol content limits in application in foods are set at 600 ppm for free gossypol and 12,000 ppm for total gossypol respectively.

With expanded use of ULGCS for human nutrition either directly as food or indirectly as feed, the cotton plant can potentially become a dual-purpose crop that will be cultivated not only as a source of natural fiber, but just as much for its seed to be used as a source of oil as well as protein. ULGCS makes available a vast source of protein without bringing additional land under the plow or an increase in the input costs.

Accordingly, there is a need for a method to remove free and bound gossypol from one or more plant parts to overcome, or at least ameliorate, one or more of the disadvantages mentioned above.

Accordingly, there is a need to provide a protein isolate that overcomes, or at least ameliorates, one or more of the disadvantages mentioned above.

Summary

There is provided a method of removing gossypol from one or more plant parts, comprising incubating a mixture of the one or more plant parts with an acidified amino acid solution, wherein amino acid comprises more than one nitrogen-containing functionality.

In one aspect, there is provided a method of removing gossypol from one or more plant parts, comprising incubating a mixture of the one or more plant parts with an acidified amino acid solution, wherein amino acid is methionine, serine, comprises more than one nitrogen-containing functionality or a mixture thereof.

Advantageously, the amino acid in the acidified amino acid solution may undergo iminization with free gossypol to result in amino acid-conjugated gossypol, which may be removed in subsequent steps. Further advantageously, the acidified amino acid solution accelerates hydrolysis of protein bound gossypol to result in dissociation of free gossypol from the protein, which then undergoes iminization with the amino acid in the acidified amino acid solution.

Further advantageously, the present method may reduce the total gossypol content in the plant parts to a greater extent as compared to a method comprising incubating the plant parts with an acidified or alkaline solution without an amino acid. Further advantageously, the present method may convert a greater amount of protein bound gossypol to amino acid-conjugated gossypol as

compared to a method comprising incubating the plant parts with an acidified solution without an amino acid.

Further advantageously, the present method results in a protein isolate which has an enriched content of an amino acid (for example, lysine) bioavailability as compared to a method which does not use an acidified amino acid solution.

Further advantageously, excess unreacted amino acid may be separated from the reaction mixture resulting from gossypol removal through anion-exchange chromatography and reused in subsequent gossypol removal reactions, thereby reducing waste production.

There is provided a protein isolate having a total gossypol content of less than 250 ppm.

Advantageously, the protein isolate has a total gossypol content of less than 250 ppm, which is under the 600 ppm maximum allowable limits per United Nations Food and Agriculture Organization and World Health Organization guidelines for food applications with gossypol content.

Definitions

The following words and terms used herein shall have the meaning indicated:

The term ‘free gossypol’ is to be interpreted broadly to include gossypol that are not bound with any proteins and is free to react and iminize with proteins or amino acids.

The term ‘bound gossypol’ is to be interpreted broadly to include gossypol that is bound with any proteins and is in an inactive and unreactive state relative to free gossypol.

The term ‘total gossypol content’ is to be interpreted broadly to include the total content of both protein bound gossypol and free gossypol, that is, non-protein bound gossypol. This term may be in reference to the remaining total gossypol content left in the target protein after gossypol detoxification, or in reference to the total gossypol content removed from the target protein during gossypol detoxification.

The term ‘detoxification’, which can be used interchangeably with the term ‘gossypol removal’, is to be interpreted broadly to include the employment of any method(s) of protein treatment(s) that is intended to reduce the total gossypol content, free gossypol content or bound gossypol content of the protein.

The term ‘protein isolate’ is to be interpreted broadly to include any protein extract that has completed the gossypol detoxification method process.

The word “substantially” does not exclude “completely” e.g. a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated

Claims

1. A method of removing gossypol from one or more plant parts, comprising incubating a mixture of the one or more plant parts with an acidified amino acid solution, wherein amino acid is methionine, serine, comprises more than one nitrogen-containing functionality or a mixture thereof.

2. The method of claim 1, wherein the acidified amino acid solution comprises an acid, the amino acid and a solvent.

3. The method of claim 2, wherein the acid is phosphoric acid, acetic acid, oxalic acid, methanoic acid, ethanoic acid, benzoic acid, citric acid, sulfuric acid, hydrochloric acid or mixtures thereof.

4. The method of any one of claims 1 to 3, wherein the acidified amino acid solution has an acid concentration of 0.1 M to 1 M.

5. The method of any one of claims 1 to 4, wherein the amino acid comprising more than one nitrogen-containing functionality is selected from the group consisting of lysine, arginine, histidine and mixtures thereof.

6. The method of any one of claims 1 to 5, wherein the nitrogen-containing functionality is an amine or a guanidine.

7. The method of any one of claims 1 to 6, wherein the acidified amino acid solution has an amino acid concentration of 10 mM to 100 mM.

8. The method of any one of claims 2 to 7, wherein the solvent is an organic solvent, ethanolamine, ammonia, water or mixtures thereof.

9. The method of claim 8, wherein the organic solvent is ethanol, methanol, isopropanol, butanol, acetone or acetonitrile.

10. The method of any one of claims 2 to 9, wherein the solvent is a mixture of organic solvent and water in a ratio of 10:90, 30:70, 50:50, 70:30, 75:25, 80:20, 85:25, 90:10, 95:5 or 99:1.

11. The method of any one of claims 1 to 10, wherein the plant parts are seeds, roots, stems, leaves, husks, hulls, flower buds or mixtures thereof.

12. The method of any one of claims 1 to 11, wherein the plant parts come from a plant, where the plant is one that produces gossypol.

13. The method of claim 12, wherein the plant is cotton, okra, soybean, or sunflower.

14. The method of any one of claims 1 to 13, wherein the incubating is undertaken at a temperature of 30 ºC to 90 ºC.

15. The method of any one of claims 1 to 14, wherein the incubating is undertaken for a time period of 30 minutes to 150 minutes.

16. The method of any one of claims 1 to 15, wherein the method further comprises the following steps:

(i) separating a supernatant of a reaction mixture as a result of incubating the mixture of the one or more plant parts with the acidified amino acid solution, and

(ii) subjecting the supernatant of (i) to anion-exchange chromatography, size exclusion chromatography or ultrafiltration.