bond to form cystine ($SCH_2CH(NH_2)CO_2H)_2$. Cystine has low solubility in water and readily precipitates out of solution. As a result, it has not been possible to produce a shelf-stable, liquid cell culture medium containing concentrations of tyrosine and cysteine required for maximal cell growth and/or protein production.

Applicants have also tried to address this problem by using less than the desired concentration of tyrosine or cysteine in the aqueous cell culture medium. In so doing, it is possible to achieve an acceptable aqueous shelf life. However, this is accomplished at the expense of optimal cell growth, protein production or viral production. In other words, cell culture medium with lower concentrations of tyrosine and cysteine supports reduced cell growth and/or protein production as compared to cell culture medium with the optimal concentrations of tyrosine and cysteine.

Thus, there exists a current need for a medium, concentrated medium or concentrated feed supplement containing amounts of tyrosine and cysteine sufficient to support maximal cell growth and/or protein or viral production while avoiding the problems caused by the limited solubility of tyrosine or the limited stability of cysteine, including the tendency of cysteine to precipitate out of solution over time. In addition, there exists a need for such a feed supplement to be in a concentrated form, so that its addition would not greatly add volume to the final cell culture system. There also exists a need that when such nutrient feeds are added, the pH and osmolality of the resulting system be automatically balanced, and that the medium or concentrated feed be available in a liquid or a dry format. Lastly, there exists a need for a single-regimen, concentrated feed supplement comprising amounts of cysteine and tyrosine sufficient to support maximal cell growth and/or protein production, and/or expressed protein of high quality.

## SUMMARY

The compositions of the disclosure are directed, in part, to cell culture media, concentrated media, or concentrated feed supplements comprising concentrations of cysteine and tyrosine that support maximal cell growth and/or protein, or viral production while avoiding the problems caused by their limited solubility and stability.

In one aspect, there is provided a serum-free feed comprising small peptides having six or fewer amino acids, wherein at least one of the amino acids in said small peptides is a cysteine and at least one of the amino acids in said small peptides is a tyrosine, and at least one of the remaining amino acids is selected from the group consisting of alanine, serine, glycine, valine, proline, aspartic acid, and glutamic acid, and wherein said feed does not contain a hydrolysate or a fraction thereof.

In another aspect, there is provided a serum-free feed for use in cell culture comprising:

a) a peptide having six or fewer amino acids, wherein at least one of said amino acids comprises cysteine; and

b) a peptide having six or fewer amino acids, wherein at least one of said amino acids comprises tyrosine.

In another aspect, there is provided a method of preparing a serum-free feed, comprising combining at least one small peptide disclosed herein with a carbohydrate and at least one amino acid or salt thereof.

In another aspect, there is provided a kit when used for the cultivation of a cell *in vitro,* said kit comprising one or more containers, wherein a first container contains a serum-free feed disclosed herein, and wherein said feed supports the growth of a eukaryotic cell in culture and/ or the expression of recombinant protein, a virus or a viral particle in said culture.

In another aspect, there is provided use of a serum-free feed disclosed herein or a kit disclosed herein, for culturing a cell, and/or to produce a recombinant protein or a virus, wherein said cell is selected from the group consisting of an insect cell, a nematode cell, a CHO cell, a COS cell, a VERO cell, a BHK cell, an AE-1 cell, a SP2/0 cell, a LS .1 cell, a PerC6 cell and a hybridoma cell.

In another aspect, there is provided a eukaryotic cell culture system comprising a serum-free feed disclosed herein, wherein the cell culture system supports increased cell growth, increased cell viability or increased protein or virus production compared to a cell culture system not containing said feed.

In one embodiment, the media, concentrated feed supplements, or concentrated media may be serum-free compositions. In one embodiment, the compositions may comprise human serum components, like human serum albumin. In a further aspect, the human serum albumin may be recombinant, (r- human serum albumin), derived from recombinant sources,

5 in

CLAIMS

1. A serum-free feed comprising small peptides having six or fewer amino acids, wherein at least one of the amino acids in said small peptides is a cysteine and at least one of the amino acids in said small peptides is a tyrosine, and at least one of the remaining amino acids is selected from the group consisting of alanine, serine, glycine, valine, proline, aspartic acid, and glutamic acid, and wherein said feed does not contain a hydrolysate or a fraction thereof.

2. A serum-free feed for use in cell culture comprising:

a). a peptide having six or fewer amino acids, wherein at least one of said amino acids comprises cysteine; and

b). a peptide having six or fewer amino acids, wherein at least one of said amino acids comprises tyrosine.

3. A serum-free feed according to claim 1 or 2, wherein at least one of the amino acid is selected from the group consisting of the twenty amino acids.

4. A serum-free feed according to any one of claims 1 to 3, wherein the feed is a single part feed.

5. A serum-free feed according to any one of claims 1 to 4, wherein the concentrated feed is at a neutral pH.

6. A serum-free feed according to any one of claims 1 to 5, wherein the feed increases the titer of a recombinant protein produced in a cell culture system.

7. A serum-free feed according to any one of claims 1 to 6, wherein the feed increases the viable cell density of a cell culture system.

8. A serum-free feed according to any one of claims 1 to 7, wherein the concentrated feed is a liquid solution, a dry powder or a granulated dry powder.

9. A serum-free feed according to any one of claims 1 to 8, wherein the small peptides can be present at concentrations of about 1 g/L to about 16 g/L, preferably about 2.5 g/L to about 8.5 g/L.

10. A serum-free feed according to claim 8 or 9, wherein the liquid solution remains free of precipitate for over 12 months when stored at 2-8°C.

11. A method of preparing a serum-free feed, comprising combining at least one small peptide according to claim 1 or 2 with a carbohydrate and at least one amino acid or salt thereof.

12. A method according to claim 11, further comprising a vitamin, a salt, a buffering agent, an inorganic element and optionally an animal-origin free peptide.

13. A method according to claim 11 or 12, wherein the feed is added to a fed-batch culture, a bioreactor or a fermenter.

14. A kit when used for the cultivation of a cell *in vitro,* said kit comprising one or more containers, wherein a first container contains a serum-free feed according to any one of claims 1 to 10, and wherein said feed supports the growth of a eukaryotic cell in culture and/ or the expression of recombinant protein, a virus or a viral particle in said culture.

15. Use of a serum-free feed according to any one of claims 1 to 10 or a kit according to claim 14, for culturing a cell, and/or to produce a recombinant protein or a virus, wherein said cell is selected from the group consisting of an insect cell, a nematode cell, a CHO cell, a COS cell, a VERO cell, a BHK cell, an AE-1 cell, a SP2/0 cell, a LS .1 cell, a PerC6 cell and a hybridoma cell.

16. A eukaryotic cell culture system comprising a serum-free feed according to any one of claims 1 to 10, wherein the cell culture system supports increased cell growth, increased cell viability or increased protein or virus production compared to a cell culture system not containing said feed.