(12) STANDARD PATENT (11) Application No. **AU 2012211969 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Polyphenol production by Vaccinium myrtillus cell cultures

(51) International Patent Classification(s)
A01N 65/00 (2006.01) ***A61K 36/45*** (2006.01)

(21) Application No: **2012211969** (22) Date of Filing: **2012.02.06**

(87) WIPO No: **WO12/106723**

(30) Priority Data

(31) Number	(32) Date	(33) Country
61/439,675	**2011.02.04**	**US**

(43) Publication Date: **2012.08.09**
(44) Accepted Journal Date: **2016.04.21**

(71) Applicant(s)
DianaPlantSciences, S.A.S.

(72) Inventor(s)
Yoon, Sung-Yong;Lall, Sonia

(74) Agent / Attorney
Griffith Hack, GPO Box 1285, Melbourne, VIC, 3001

(56) Related Art
D1 : MADHAVI ET AL: "Isolation of bioactive constituents from Vaccinium myrtillus (bilberry) fruits and cell cultures". Plant Science, 1998, vol. 131, pages 95-103
D2 : YAO ET AL: "Protective activities of Vaccinium antioxidants with potential relevance to mitochondrial dysfunction and neurotoxicity". NeuroToxicology, 2007, vol. 28, pages 93-100

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 August 2012 (09.08.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/106723 A1

(51) International Patent Classification:
A01N 65/00 (2009.01) *A61K 36/45* (2006.01)

(21) International Application Number:
PCT/US2012/024008

(22) International Filing Date:
6 February 2012 (06.02.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/439,675 4 February 2011 (04.02.2011) US

(71) Applicant *(for all designated States except US)*: **DIANA-PLANTSCIENCES, INC.** [US/US]; 18183 SW Boones Ferry Road, Portland, OR 97224 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **YOON, Sung-Yong** [KR/US]; 5474 Bay Creek Drive, Lake Oswego, OR 97035 (US). **LALI, Sonia** [IN/US]; 14604 NW Wendy Lane, Portland, OR 97229 (US).

(74) Agents: **BROWN, Perry, N.** et al.; Workman Nydegger, 1000 Eagle Gate Tower, 60 East South Temple, Salt Lake City, UT 84111 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2012/106723 A1

(54) **Title**: POLYPHENOL PRODUCTION BY VACCINIUM MYRTILLUS CELL CULTURES

(57) **Abstract**: Cell cultures of Vaccinium myrtillus configured to grow in suspension culture in a liquid medium. The cells are derived from one or more V. myrtillus plant parts, such as an edible plant part (e.g., a leaf part or a berry part) or a stem part. The cells are adapted to grow to a high density is a relatively short period of time (e.g., about 7 days). In addition, the cells are adapted to produce high concentrations of polyphenols and/or procyanidins and essentially no anthocyanin. Methods for production of polyphenols and/or procyanidins from Vaccinium myrtillus cells grown in suspension culture are disclosed.

POLYPHENOL PRODUCTION BY *VACCINIUM MYRTILLUS* CELL CULTURES

BACKGROUND

[0001] The extract from the fruit *Vaccinium myrtillus* (more generally referred to as bilberry) has long been used for therapeutic purposes. In Europe it has been used for hundreds of years to treat diarrhea and dysentery, as well as diseases of the lungs, liver, and stomach. In addition, it is believed that British fighter pilots in World War II ate bilberry jam to help improve their night vision. More recently, extracts from the fruit of the *V. myrtillus* plant has been shown to possess potential anti-carcinogenic activity. The clinical benefits of *V. myrtillus* as both a dietary supplement and a therapeutic have been attributed to the presence of abundant amounts of flavonoids and anthocyanins in Bilberry. These antioxidant compounds scavenge damaging particles known as free radicals in the body, helping to prevent or reverse damage to cells. Antioxidants have been shown to help prevent a number of long-term illnesses such as heart disease, cancer, and macular degeneration. The *V. myrtillus* fruit also contains tannins, which are known to act as both an anti-inflammatory and an astringent.

[0002] Polyphenols are widely distributed in plants, fruits, and vegetables and have received considerable attention because of their physiological functions in human and animal health, including antioxidant, antimutagenic and cancer prevention activities (Salvia *et al., J. Agric. Food Chem.* 39: 1549-1552, 1991; Bomser *et al.*, *Cancer Lett.*, 135: 151-157, 1999; Zhao *et al.*, *Carcinogenesis*, 20: 1737-1745, 1999). Epidemiological studies have suggested that flavonoids, among the polyphenols, may reduce the risk of heart disease (Hertog *et al.*, *Lancet*: 342: 1007-1011, 1993). Additionally, dietary flavan-3-ols and/or proanthocyanidins have been shown to reduce the incidence of atherosclerosis and coronary heart disease in experimental animals (Tijburg *et al.*, *Atherosclorosis*, 135: 37-47, 1997; Yamakoshi *et al.*, *Atherosclerosis*, 142: 139-149, 1999). One of the mechanisms responsible for these effects involves their inhibition of oxidation of low density lipoprotein (LDL) (Steinberg, *Circulation*, 85: 2337-2344, 1992).

[0003] Berries of the *Vaccinium* species have been shown to possess radical scavenging capacity in various in vitro models using assays of the oxygen radical absorbance capacities (ORAC), the ferric reducing antioxidant power (FRAP), the total

oxidant scavenging capacity (TOSC), and the free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as well as antioxidant capacities in inhibiting oxidation of methyl linoleate, liposomes, and human low-density lipoprotein (LDL) (Maata-Riihinen et al). Cultivated cranberry (*V. macrocarpon* Ait.) and wild lingonberry contain both A- and B-type procyanidins (Gu *et.al.*, Morimoto *et.al.*, Foo *et.al.*) whereas primary B-type procyanidins were identified in wild (*V. angustifolium* Ait.) and cultivated blueberries (*V. corymbosum* L., *V. ashei* L.) (Foo *et.al.*; Prior *et. al.*; Schmidt *et.al.*) Rare A-type low molecular weight procyanidins were detected in wild lingonberry, cranberry, bilberry, and bog whortleberry and were present at higher levels than the more common B-type procyanidins (Maata-Riihinen *et.al.*). The rare A-Type procyanidin is known to act as deterrent to adhesion of bacterial cilia to the endothelial layers helping in prevention of urinary tract infection (Nowack and Schmidt; Foo *et al*[2];). It is a general anti inflammatory agent which is known in Bilberry for many decades to cure intestinal inflammations. In addition, *V. myrtillus* leaves have 35 different flavon-3-ols, procyanidins, flavonols and their glycosides, and various phenolic acid conjugates (Hokannen *et.al.*).

[0004] *Vaccinium myrtillus* is difficult to grow and is therefore rarely cultivated. As a result, the fruit is generally collected from wild plants during its limited growing season (May through September), which must be both wet and warm. Thus, the supply of the berries is unreliable and the berries are available in limited quantities. Moreover, the fruit are softer and juicier than the related blueberry, such that they must be harvested by hand, and are difficult to transport, which contribute to the high cost of the fresh fruit harvested from the *V. myrtillus* plant. Also due to the high demand of the ripe fruit, unripe fruits and leaves are not economically viable products to collect. These are the parts of the plant that have highest amounts of the procyanidin. In view of the clinical benefits of *V. myrtillus* and the difficulty in cultivating these plants, there is a need to develop a sustainable *in vitro* culture system for the cells of these plants.

SUMMARY

[0005] The present disclosure relates to cell culture of *Vaccinium myrtillus* that are configured to grow in suspension culture in a liquid medium. The cells are derived from one or more *V. myrtillus* plant parts, such as an edible plant part (e.g., a leaf part or a berry part) or a stem part. The cells are adapted to grow to a high density in a relatively

short period of time (e.g., about 7 days). In addition, the cells are adapted to produce high concentrations of polyphenols and/or procyanidins and essentially no anthocyanin. Methods for production of polyphenols and procyanidins from *Vaccinium myrtillus* cells grown in suspension culture are also disclosed.

[0006] In one embodiment, a cell culture is described. The cell culture includes a plurality of friable *Vaccinium myrtillus* cells in a suspension cell culture. The cells in suspension culture are derived from one or more of: a hypocotyl, a cotyledon, a leaf section, a stem section, or a root section of a seedling; or a berry, a stem section including a node or an internode, or a leaf section of a mature plant. In one embodiment, the cells can be derived from an edible plant part, such as a leaf part or a berry part. The cells are selected to be capable of obtaining a packed cell volume of at least 55% in 7 days of growth, wherein at least 10% of a dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of polyphenols and at least 5% of a dry mass of the plurality of Vaccinium myrtillus cells is comprised of procyanidins.

[0007] Preferably, at least 12.5%, 15%, 20%, or more of the dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of polyphenols. Preferably, at least 7.5%, 10%, 15%, 20%, or more of the dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins. It is also preferred that the mass of cells is essentially free of anthocyanins. For example, it is preferred that the dry mass of the plurality of *Vaccinium myrtillus* cells includes less than 0.5%, 0.1%, 0.01%, 0.001%, or less anthocyanin.

[0008] In another embodiment, a method of producing a cell culture of *Vaccinium myrtillus* cells is described. The method includes (1) producing a cell callus of Vaccinium myrtillus cells derived from one or more of: a hypocotyl, a cotyledon, a leaf section, a stem section, or a root section of a seedling; or a berry, a stem section including a node or an internode, or a leaf section of a mature plant, (2) introducing one or more cells derived from the callus into a liquid medium, (3) agitating the one or more cells in the liquid medium, (4) replacing the liquid medium with a fresh liquid medium or transferring the cells to fresh a fresh liquid medium to establish a suspension cell culture of Vaccinium myrtillus, (5) growing the suspension cell culture of Vaccinium myrtillus to a packed cell volume of at least 55%, and (6) selecting suspension cell cultures having at least 10% of a dry mass of the plurality of *Vaccinium myrtillus* cells comprised of polyphenols and/or at least 5% of a dry mass of the cells comprised of procyanidins.

[0009] In yet another embodiment, a method of increasing growth of *Vaccinium myrtillus* cells in suspension cell culture is described. The method includes (1) providing a suspension cell culture of Vaccinium myrtillus cells, (2) culturing the cells in a liquid medium in suspension culture, and (3) selecting suspension cell cultures having greater than 45% packed cell volume (PCV).

[0010] In one embodiment, the method of increasing growth of *Vaccinium myrtillus* cells in suspension cell culture further includes selecting suspension cell cultures having increased polyphenol and procyanidin accumulation in response to increased sugar concentration in the liquid medium. In one embodiment, the sugar concentration in the liquid medium includes approximately 30-60 g/L sucrose. In one embodiment, procyanidin accumulation in the cells in suspension culture is increased from about 1-2 g/L of PCV at 20 g/L sucrose to about 3-7 g/L of PCV at 30 g/L sucrose. In one embodiment, polyphenol accumulation in the cells in suspension culture is increased from about 2-4 g/L of PCV at 20g/L sucrose to about 5-10 g/L of PCV at 60 g/L sucrose.

[0011] In still yet another embodiment, a method of increasing polyphenol production from *Vaccinium myrtillus* cells in culture is described. The method includes (1) selecting a plurality of *Vaccinium myrtillus* cells adapted to grow in suspension culture, (2) and culturing the cells in suspension culture in the presence of a sufficient amount of sugar to increase polyphenol production.

[0012] In one embodiment, the sufficient amount of sugar is the liquid medium having greater than 20 g/L sugar, 20 g/L to 30 g/L sugar, or greater than 30 g/L sugar. In one embodiment, the sugar is sucrose. In another embodiment, the sugar is glucose. In one embodiment, the sugar is present in an amount sufficient for polyphenol production to increase above 3 g/L packed cell volume (PCV). In another embodiment, the sugar is present in an amount sufficient for polyphenol production to increase to at least 7 g/L packed cell volume (PCV).

[0013] In still yet another embodiment, a method of extracting polyphenols from *Vaccinium myrtillus* cells in culture is described. The method includes (1) selecting a plurality of Vaccinium myrtillus cells adapted to grow in suspension culture, and (2) extracting polyphenols from the cells using a solvent, wherein at least 10% of a dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of polyphenols and at least 5% of a dry mass of the plurality of Vaccinium myrtillus cells is comprised of procyanidins.

[0014] In one embodiment, the solvent includes acetone, acetic acid, and water. In one embodiment, the solvent includes 70% acetone (v/v) and 0.5% acetic acid (v/v).

The present invention as claimed herein is described in the following items 1 to 25:

1. A cell culture, comprising:

a plurality of friable *Vaccinium myrtillus* cells growing in a suspension cell culture, the cells being derived from one or more of:

a hypocotyl, a cotyledon, a leaf section, a stem section, or a root section of a seedling; or

a berry, a stem section including a node or an internode, or a leaf section of a mature plant,

wherein the plurality of *Vaccinium myrtillus* cells are selected to be capable of obtaining a packed cell volume of at least 55% in 7 days of growth in the suspension cell culture, and

wherein at least 5% of a dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins.

2. The cell culture of item 1, wherein at least 10%, 15%, or 20% of the dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of polyphenols.

3. The cell culture of item 1 or 2, wherein at least 7.5% of the dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins.

4. The cell culture of any one of items 1 to 3, wherein at least 10% of the dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins.

5. The cell culture of any one of items 1 to 4, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.5% anthocyanin.

6. The cell culture of any one of items 1 to 5, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.1% anthocyanin.

7. The cell culture of any one of items 1 to 6, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.01% anthocyanin.

8. The cell culture of any one of items 1 to 7, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.001% anthocyanin.

9. The cell culture of any one of items 1 to 8, wherein the procyanidins comprise dimers, trimers, tetramers, pentamers, hexamers, heptamers, octamers, nonamers and decamers.

10. The cell culture of any one of items 1 to 9, wherein the production of polyphenols in the plurality of *Vaccinium myrtillus* cells is greater than 3 grams per liter of packed cells.

11. The cell culture of any one of items 1 to 10, wherein the production of polyphenols in the plurality of *Vaccinium myrtillus* cells is greater than 7 grams per liter of packed cells.

12. The cell culture of any one of items 1 to 11, wherein the plurality of *Vaccinium myrtillus* cells are selected to be capable of doubling in density within 7 days or less of growth in the suspension cell culture.

13. The cell culture of any one of items 1 to 12, wherein the liquid medium comprises a carbohydrate source, major salts, minor salts, and one or more hormones selected from the group consisting of an auxin and a cytokinin.

14. A method of increasing growth of *Vaccinium myrtillus* cells in suspension cell culture, the method comprising:

providing a suspension cell culture of *Vaccinium myrtillus* cells;

subculturing the cells in a liquid medium in suspension cell subculture; and

selecting suspension cell subcultures having greater than 45% packed cell volume (PCV) after 7 days of growth, at least 5% of a dry mass of the *Vaccinium myrtillus* cells in the selected suspension cell cultures being comprised of procyanidins, and less than 0.5% of the dry mass of the *Vaccinium myrtillus* cells in the selected suspension cell cultures being comprised of anthocyanin.

15. The method of item 14, wherein providing a suspension cell culture of *Vaccinium myrtillus* cells comprises:

producing a cell callus of *Vaccinium myrtillus* cells derived from one or more *Vaccinium myrtillus* plant parts;

introducing one or more cells derived from the callus into a liquid medium;

agitating the one or more cells in the liquid medium;

replacing the liquid medium with a fresh liquid medium or transferring the cells to fresh a fresh liquid medium to establish the suspension cell culture of *Vaccinium myrtillus*.

16. The method of item 14 or 15, wherein about 10% of the dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins.

17. The method of any one of items 14 to 16, wherein the plurality of *Vaccinium myrtillus* cells contain less that about 0.1 % anthocyanin or the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.1% anthocyanin.

18. The method of any one of items 14 to 17, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.01% anthocyanin.

19. The method of any one of items 14 to 18, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.001% anthocyanin or the plurality of *Vaccinium myrtillus* cells are substantially free of anthocyanin.

20. The method of any one of items 14 to 19, wherein the liquid medium comprises a carbohydrate source, major salts, minor salts, and one or more hormones selected from the group consisting of an auxin and a cytokinin.

21. A method of extracting polyphenols from *Vaccinium myrtillus* cells in culture, the method comprising:

selecting a plurality of *Vaccinium myrtillus* cells adapted to grow in suspension culture, the cells being derived from one or more of:

a hypocotyl, a cotyledon, a leaf section, a stem section, or a root section of a seedling; or

a berry, a stem section including a node or an internode, or a leaf section of a mature plant; and

extracting polyphenols from the cells using a solvent,

wherein at least 10% of a dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of polyphenols and at least 5% of a dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins.

22. The method of item 21, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.5% anthocyanin.

23. The method of item 22, further comprising selecting *Vaccinium myrtillus* cells having at least 10% of the dry mass of the plurality of *Vaccinium myrtillus* cells comprised of polyphenols, at least

5% of the dry mass of the *Vaccinium myrtillus* cells comprised of procyanidins, and less than 0.5% of the dry mass of the *Vaccinium myrtillus* cells comprised of anthocyanin.

24. The method of item 23, further comprising:

producing a cell callus of *Vaccinium myrtillus* cells derived from one or more *Vaccinium myrtillus* plant parts;

introducing one or more cells derived from the callus into a liquid medium;

agitating the one or more cells in the liquid medium;

replacing the liquid medium with a fresh liquid medium or transferring the cells to fresh a fresh liquid medium to establish a suspension cell culture of *Vaccinium myrtillus*;

subculturing the suspension cell culture of *Vaccinium myrtillus* cells; and

selecting suspension cell subcultures having at least 10% of the dry mass of the plurality of *Vaccinium myrtillus* cells comprised of polyphenols, at least 5% of a dry mass of the *Vaccinium myrtillus* cells in the selected suspension cell cultures comprised of procyanidins, and less than 0.5% of the dry mass of the *Vaccinium myrtillus* cells comprised of anthocyanin.

25. The method of item 24, further comprising selecting suspension cell subcultures having greater than 45% packed cell volume (PCV) after 7 days of growth.

26. A polyphenol extracted by the method of any one of items 21 to 25.

[0015] These and other features of the present disclosure will become more fully apparent from the following description and appended claims, or may be learned **by** the practice of the claims as set forth hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] To further clarify the above and other advantages and features of the present disclosure, a more particular description of the subject matter of the disclosure will be rendered by reference to specific embodiments thereof which are illustrated in the appended drawings. It is appreciated that these drawings depict only illustrated embodiments of the disclosure and are therefore not to be considered limiting of its scope.

The subject matter of the disclosure will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

[0017] Figure 1 shows the consumption of sugar with increasing biomass from 25% initial biomass to 50% in one week.

[0018] Figure 2 shows the growth (a) RI (b) and production (c) at different shaker speeds. The 500ml flasks were all inoculated at 20% PCV and PCV, RI and production yield was measured after 6 days of growth.

[0019] Figure 3 shows the HPLC chromatogram of extracts from suspension cells derived from stem, hypocotyl, leaf and cotyledon explants in fluorescence detector mode.

The labels 1 through 12 indicate the degree of polymerization of procyanidins, respectively: 1, monomers; 2, dimers; 3, trimers; 4, tetramers; 5, pentamers; 6, hexamers; 7, heptamers; 8, octamers; 9, nonamers.

[0020] Figure 4 shows the HPLC chromatogram of extracts from suspension cells of Bilberry and cocoa in fluorescence detector mode. The labels 1 through 12 indicate the degree of polymerization of procyanidins, respectively: 1, monomers; 2, dimers; 3, trimers; 4, tetramers; 5, pentamers; 6, hexamers; 7, heptamers; 8, octamers; 9, nonamers.

This figure confirms that the peaks in Bilberry are procyanidin oligomers by the fact that the 2 cell lines were extracted in the same way and are run under same conditions and they have same retention time for each oligomer.

[0021] Figure 5 shows a UV absorption pattern at 280 nm of cocoa (a) and bilberry (b) extracts confirming the presence and detection of procyanidins in Bilberry.

DETAILED DESCRIPTION

[0022] The present disclosure relates to cell culture of *Vaccinium myrtillus* that are configured to grow in suspension culture in a liquid medium. The cells are derived from one or more *V. myrtillus* plant parts, such as an edible plant part (e.g., a leaf part or a berry part) or a stem part. The cells are adapted to grow to a high density is a relatively short period of time (e.g., about 7 days). In addition, the cells are adapted to produce high concentrations of polyphenols and/or procyanidins and essentially no anthocyanin. The subject matter of the disclosure will be described and explained with additional specificity and detail through the use of the following Examples.

EXAMPLES

Example 1. Surface sterilization and seed germination

[0023] *Vaccinium myrtillus* seeds were obtained from Horizon Herbs, Oregon. Leaves, stem sections and immature berries of *V. myrtillus* (Erin's Bilberry) used in this Example and the Examples below were collected from National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon.

[0024] Leaves, stem sections, and immature berries were rinsed in running water for 20 minutes and rinsed in 75% ethanol for 1 minute. Stems were then cut into smaller pieces. Then the stems, leaves and immature berries were washed in 25% sodium hypochlorite (v/v) for 15 minutes followed by 5 rinses in sterile distilled water.

[0025] Seeds (Horizon Herbs, Oregon) were surface sterilized by rinsing first, in 75% ethanol for 1 minute. Then they were washed in 25% sodium hypochlorite (v/v) for 15 minutes followed by 5 rinses in sterile distilled water. Seeds were then suspended in 0.1% agarose and plated onto 100 x 25 mm Petri plates (approximately 100 seeds per plate). They were germinated on MS (Murashige and Skoog) medium (4.43 g/L) with 7g/L agar under a 16 hour light and 8 hour dark photoperiod at 23 °C for 4 weeks.

Example 2. Callus Induction from *Vaccinium myrtillus* Seedlings Grown *In Vitro*

[0026] More proliferative growth and friable callus are very important characteristics of a successful cell line. This example describes methods and media conditions which were optimized to initiate and maintain callus from various explants derived from *in vitro* grown *V. myrtillus* seedlings.

[0027] Callus was initiated from hypocotyls, cotyledons, leaves, stem sections and roots of *in vitro* grown seedlings. Plant parts were cut into 5mm sections. All media

were sterilized by autoclaving for 20 minutes at 121°C and 15 PSI (pounds per square inch) unless otherwise stated. All growth regulators were filter sterilized and added post autoclave unless otherwise stated. All cultures were kept in darkness at 25°C unless otherwise stated.

[0028] Explants were put on various callus induction media (Table 1). Plates were kept in darkness at 25 °C. First signs of callus formation were seen after 2 weeks of putting explants on plates with media VM1445, VM1196, VM1204, and VM1233 (VM1233 is described in Madhavi *et al.*, *Plant Science,* 131:95-103, 1998). Callus induction rates were 83%, 85%, 85% and 70% respectively. However, callus produced on media VM1196 and VM1204, both of which had 24 mM ammonium sulfate and 8 mM potassium nitrate but different base salts, (MS basal salts no nitrogen and B5 major salts modified, respectively; Table 1)was softer than callus produced on medium with 1mM ammonium sulfate and 24 mM potassium nitrate (VM1445). Callus produced on medium VM1233 (Madhavi *et al.*, *Plant Science,* 131:95-103, 1998) was very compact and non proliferative. Madhavi *et al.* showed that callus was subcultured on this medium for three subculture periods each at three week intervals, although the quality of the callus on this medium was not discussed in that reference. Subculturing callus using the conditions of Madhavi *et al.* produced the same initial results as those described in the reference, but it was noted that with every subculture the callus became hard and non proliferative. Thus, the quality of the callus using medium VM1233 decreased with every subculture. The removal of the polyvinylpyrrolidone (PVP; medium VM1204) from the VM1233 medium helped to make the callus soft. On media VM1491 and DC1152, 50% and 10% of the explants produced sustainable callus, respectively. Explants and calli were transferred to fresh medium every 3 weeks. Once the calli were separated from explants, calli that were very proliferative were subcultured every 2 weeks. Fast growing cell lines were chosen for subculture. Continuous subculture helped change the morphology of the callus to a more desirable friable morphology.

[0029] Subculturing continuously on medium with higher ammonium sulfate and lower potassium nitrate resulted in the callus becoming very brown from the stress and it eventually stopped growing. This was evident from the fact that medium VM1445, which had full strength Gamborg's B5 (B5) medium, but did not have ammonium sulfate or potassium nitrate did not show browning and eventual death. Media VM1196 and

VM1204 were discontinued after 9 weeks because of undesired browning of the callus. Various media (Table 1) were tried in order to characterize a medium that would support growth of callus sustainably, without browning and eventual death. Medium VM1516 which had full strength MS salts showed very proliferative and sustainable growth. When VM1445 and VM1516 were compared, VM1516 gave the most proliferative calli and also helped change the morphology from compact to granular and eventually friable callus. Medium VM1516 also proved the best for sustainably maintaining callus derived from *V. myrtillus* seedlings. VM1516 was also confirmed to be the best medium for initiating new callus from various *V. myrtillus* seedling explants, with a success rate of 83%.

Example 3. Callus induction from *Vaccinium myrtillus* tissue collected from NCGR

[0030] This example describes methods and media formulations for initiating and maintaining callus from various explants (derived from berries, nodes, internodes, or leaves) derived from field-grown *V. myrtillus* plants.

[0031] Mature leaves and stems, and immature berries were surface sterilized, as discussed above. The plant parts were cut into small 5 mm sections before explanting into media VM1516 and VM1491. Berries were cut open under sterile conditions and the skin was placed on culture plates with media. Any berry flesh was removed before explanting.

[0032] Culture plates were kept in darkness at 25°C. In general, callus was observed 4 weeks after initially explanting materials on VM1516 and 6 weeks on VM1491. With regard to leaf explants, there was a 53% callus induction rate overall. Callus from leaf explants was produced in VM1491 (73% of initial explants) and in medium VM1516 (76% of initial explants) and no callus was observed in medium VM1672 and TC1596. It was observed that the callus produced in VM1516 was more vigorous than that produced in VM1491. With regard to nodes, 47% of those explants produced callus in VM1516. Internodes were placed on 3 different media VM1516, VM1491 and TC1596. On VM1516, 51% explants produced callus, while only 20% produced callus on VM1491 and none on TC1596. Among the explants from berries, 59% produced callus on VM1516.

[0033] Callus derived from *V. myrtillus* tissue was subcultured every 3 weeks on VM1516. This callus was very proliferative and friable cell lines were selected for

further maintenance. Calli derived from these tissues were maintained on medium VM1516 for over eight months and have demonstrated consistent proliferation without change in quality of the callus.

Example 4. Suspension Creation from Callus Derived from *Vaccinium myrtillus* Seedlings

[0034] Friable cell lines created as in example 2 were chosen for initiation of suspensions. Cell suspensions were created by introducing 1g (approx) of fresh 2 week old *V. myrtillus* seedling callus (prepared as in Example 2) into 15 ml of liquid medium (VM1799, VM1831 or DC1151; Table 2) in a sterile 125 ml Erlenmeyer flask. The flasks were covered with sterile silicon (foam) caps and agitated at 120 revolutions per minute (rpm) in a gyrotatory shaker. The suspensions were kept in darkness at 23°C. To establish the cell culture, the spent medium was removed and fresh medium was added every week for 2 subcultures. The growth of cells was measured by the rate of carbohydrate consumed by measuring the delta of refractive index (RI) (as measured by degrees of BRIX (i.e., % BRIX)) of the medium. If the RI was less than or equal to half of the initial RI of the medium, fresh medium was added to the cells. If the RI was greater than half, fresh medium was only added after 2 weeks. The subcultures were transferred weekly or biweekly as deemed necessary.

[0035] Cultures that formed as either granular or fine suspension of cells were retained, while cultures that did not form suspension cultures were discarded. Once the suspension culture was established (3-4 subculture periods), 25-35% of the cells were transferred to flask with fresh medium every week. Packed cell volume (PCV) and RI was recorded at every subculture to measure cell growth.

[0036] Sustainable stable suspensions were obtained within 6 subcultures of initiating suspensions from callus.

Example 5. Suspension Creation from Callus Derived from *Vaccinium myrtillus* tissue collected from NCGR

[0037] Friable cell lines were chosen for initiation of suspensions. Cell suspensions were created by introducing *V. myrtillus* callus (prepared as in Example 3 from nodes, internodes, leaves, and berries) into liquid medium (VM1933; Table 2) in sterile Erlenmeyer flasks. The flasks were covered with sterile silicon (foam) caps and agitated at 120 revolutions per minute (rpm) in a gyrotatory shaker. The suspensions were kept in

darkness at 25°C. To establish the cell culture, the spent medium was removed and fresh VM1933 medium was added. The growth of cells was measured by the rate of carbohydrate consumed by measuring the delta of refractive index (RI) of the medium. If the RI was less than or equal to half of the initial RI of the medium, fresh medium was added to the cells. If the RI was greater than half, fresh medium was only added after 2 weeks.

Example 6. Optimization of Cell Growth

[0038] This example describes methods used to increase cell growth of suspensions. Cell culture productivity increases as a function of the rate of cell growth and the density at which cell growth stops. To determine the optimal inoculation density, suspension cultures of *Vaccinium myrtillus* cells were initiated with an inoculum size yielding a starting cell density of 15% packed cell volume ("PCV") and 25% PCV and allowed to grow for 7 days. Cultures initiated at a cell density of 15% PCV did not reach maximal density within 7 days. Cultures initiated at a cell density of 25% PCV in Medium VM1831 (Table 1.) doubled in density (i.e., total cell volume) within 7 days and reached a maximal average cell density of 45-50% PCV within 7 days with some cell line cultures showed over 55-60% PCV at day 7. Cell selection helped to capture cultures that reached a 45% PCV or more PCV within 7 days or less (a rapidly growing cell culture). Cultures that took more than 7 days to reach 45% PCV were discarded.

[0039] After careful selection of cells that showed high sugar consumption and good growth (Fig 1.) through a number of generations, it was seen that the final cell density on day 7 was very high (PCV around 65.8 ± 0.63) when flasks were initiated with a 25% inoculum. This inhibited proper shaking of the cells in the 125 ml flasks with a working volume of 40 ml. Therefore, optimization of inoculums size was required again once the cell line improved through cell selection process. Calculation of the doubling time indicated that a 20% PCV inoculums would yield around 60% final PCV on day 7. Experimental data supported this and showed there was a significant difference ($P = 0.008$) in the final PCV (59.9 ± 0.77) but no deleterious effect ($P = 0.39$) on production or productivity. Hence the inoculums size was changed to 20% PCV.

Example 7. Optimization of Polyphenol production from Bilberry suspensions by Cell Selection and Medium Optimization of Suspension Cell Culture

[0040] After optimization of growth, production of polyphenol production was

achieved by changing media formulation and additional criteria for cell selection.

[0041] The carbohydrate consumption was rapid in the cultures with the cultures reaching RI of 0 to 0.6 by day 7. Polyphenol and/or procyanidin production in VM1831 was low possibly due to sugar starvation. The medium VM1831 had 20g/L of sucrose. Liquid media was optimized by adjusting carbohydrate level to maintain cultures without nutrient starvation. A new medium VM1933 (Table1.) was formulated with 30g/L of sucrose to avoid sugar starvation of the cells. In this medium the RI went down to between 0.8 and 1.0. The production values of polyphenols went up from about 2-4 g/L of PCV at 20g/L sucrose to about 5-10 g/L of PCV at 60 g/L sucrose within 4 subcultures and could be maintained at a high production level. The production values of procyanidins went up from about 1-2 g/L of PCV at 20 g/L sucrose to about 3-7 g/L of PCV at 30 g/L sucrose within 4 subcultures and could be maintained at a high production level.

[0042] The cell selection process where we selected for flasks that produced higher than average polyphenols quantified by a high through at each subculture allowed for further improvement in polyphenol and procyanidin production levels.

Example 8. Detection and confirmation of polyphenol and procyanidin production in suspensions from various parts of bilberry seedlings

[0043] In this example we demonstrate that we have been able to produce procyanidin from suspensions prepared as in examples 4 and 5 from all parts (roots, hypocotyls, berries, cotyledons, stem and leaves) of the plants. Figures 3 shows the chromatogram showing various sources. We have also been able to confirm that what we are seeing is procyanidins by overlaying with confirmed cocoa procyanidin chromatograms (Fig 4) that show same retention times for each oligomer as in cocoa, which also show additional isomers of dimer, trimer and tetramer in Bilberry. Also running a UV absorption at 280nm showed that the pattern was similar to cocoa and also confirmed presence of procyanidin (Fig 5).

Example 9. Extraction of Polyphenols from *Vaccinium* Callus Culture and Suspension Cultures

[0044] This example describes methods developed for extracting polyphenols from callus and suspension cells of *Vaccinium* cultures developed in examples 1-5. Polyphenols were extracted from approximately 0.4 ml of fresh cells from suspensions

with 0.4 ml 70% (v/v) acetone with 0.5% acetic acid. A robust high throughput method was used as follows: From each flask of cell culture to be analyzed, the packed cell volume (PCV) of the sample was recorded prior to transferring 0.4 ml into a 96- deep well plate. The supernatant from each well was removed and discarded with a plastic transfer pipette. Next, 0.4 ml of extraction solvent (70% acetone, 29.5% water, 0.5% acetic acid) and a tungsten carbide bead were added to each well, and the plate was placed on a Mixer Mill to grind the cells at 18 Hz for 4 minutes. The plate was then placed in a centrifuge and centrifuged at 6000 rpm for 4 minutes to separate the cells from the extract.

Example 10. Preliminary Analysis of Polyphenol Production in Culture

[0045] The method used to carry out the procyanidin analysis reaction was designed to approximate fairly closely the original Swain and Hillis (*J. SCI. Food Agric.* 10:63, 1959) method and Porter *et al.* (*Phytochemistry*, 25(1):223, 1986) method. The butanol-HCl extraction assay was used to measure polyphenols in the extracts of *Vaccinium myrtillus* suspended cells. The polyphenols are hydrolyzed to the monomers of (-)-epicatechin and cyanidin by combining 0.1 ml of aqueous acetone extract and 1.0 ml of butanol-HCl reagent (95:5 v/v) and heating the solution at 75°C for 60 minutes in a Qiagen deep well block (Valencia, CA, USA). Presence of cyanidin in the hydrolyzed sample was observed by the formation of a pink color. The absorbance at 520 nm was determined, and procyanidin content was calculated based on the amount of cyanidin formed using a calibration curve created using different concentrations of procyanidin B2 purchased from Chromadex, Inc. (Irvine, CA). Brighter pink color indicated higher concentration of procyanidins in suspension cultures. Based on this method the procyanidin content of several suspension cultures ranged from 1g/L to 10g/L.

[0046] Total polyphenol content of bilberry cell extracts was measured using the Folin-Ciocalteau assay (Slinkard, K.; Singleton,V.L. Total Phenol Analysis:Automation and Comparison with Manual Methods. *American Journal of Enology and Viticulture* 1977, 28: 49-55). Cell culture extracts, in 70% acetone with 0.5% acetic acid, were analyzed for total polyphenol content by taking 25 µl extract and adding it to 0.975 ml of water to dilute the sample prior to beginning the assay. For the quantification of polyphenols, 20µl of the diluted extract is added to 0.790 ml water plus 50 µl of Folin-Ciocalteau reagent. The reaction is then stopped by the addition of 150 µl sodium

carbonate solution. The resulting solution is measured at 765 nm and compared to a calibration curve of various dilutions of gallic acid solution measured by the same assay to determine the concentration of total polyphenols in the cell extracts.

Example 11. Small scale extraction of polyphenols from fresh bilberry cells or ground freeze dried cells

[0047] Bilberry cells (0.5 mL) without media or 50 mg of ground bilberry cells were sampled in 2.0 ml of micro-tubes or 1.2 ml tubes in a 96 well block from Qiagen, Inc. Appropriate volumes of acidic (0-2% of citric, acetic or ascorbic) aqueous extraction solvent (30-80% of acetone, ethanol, methanol) was added to each of the bilberry cell samples and then placed into ultrasonicator or BeadMill to extract polyphenols and/or procyanidins. The samples were centrifuged for 4 minutes at 6000rpm (RCF 5996). The supernatant may be filtered with 0.45 um membrane filter and diluted to 10x (if necessary) by using the same aqueous extraction solvents prior to analysis. The leftover extracts were stored in -20 degree of freezer for further analyses.

Example 12. Analysis of Procyanidin Production in Bilberry Culture

[0048] LC analyses were performed on the Bilberry cell extracts using a Waters (Milford, Massachusetts, USA) Alliance HPLC system equipped with a CTC Analytics PAL autosampler (Leap Technologies, Carrboro, NC, USA), Waters 626 pump with 600S Controller and a Waters 2996 photodiode-array detector (PDA) scanning from 190 to 780nm. Gradient elution was carried out with water-0.1% formic acid (solvent A) and acetonitrile-0.1% formic acid (solvent B) at a constant flow-rate of 0.3 ml/ minute. A linear gradient with the following proportions *(v/v)* of solvent B was applied (t(min), %B): (0, 7), *(5, 15),* (20, *75), (25,* 100), *(35,* 100), (35.1, 7) *(45, 7).* The column was Ultra Aqueous C18 column (100 x 2.1 mm i.d., 3.5 μm) (Restek, Bellefonte, PA. USA). The procyanidin monomers of (+)-catechin, (-)-epicatechin, and oligomeric procyanidins (dimer to hexamer) were monitored at 280 nm. A Waters Quattro Micro triplequadrupole mass detector (Milford, Massachusetts, USA) was used to obtain the MS data and analyzed by MassLynx™ software. Full-scan data acquisition was performed, scanning from m/z 150 to 1800. Authentic standards for catechin, epicatechin, were purchased from Sigma-Aldrich, Inc. (St. Louis, MO) and dilutions made to create calibration curves in order to detect and quantify the metabolites.

[0049] Analysis of quantifiable procyanidins was performed by normal phase HPLC

system consisted of the Waters 2795 separation module, the Waters 996 PDA detector and the Waters 474 scanning fluorescence detector. Characterization and separation conditions of procyanidins in bilberry cell extracts obtained using Develosil Diol (250x4.6 mm ID, 5 μ particle size) adapted from Kelm et al., the improved process for analyzing for separating, and for isolating polar protic monomers and/or oligomers. (U.S. Pat. No. 0075020). The binary mobile phase consists of solvent (A), acetonitrile: acetic acid (98:2, v/v) and solvent (B), methanol: water: acetic acid (95:3:2, v/v/v). A linear gradient elution was performed at 30 °C with 0.8 mL/min flow rate as follows: 0-35 min, 100-60% A; 35-40 min, 60% A; 40-45 min, 60 - 100%A. Separations of oligomer procyanidins were monitored by fluorescence detection (excitation wavelength at 276 nm, emission wavelength at 316 nm), UV detection at 280 nm (Figure 10A). (Lazarus et al. J. Agric. Food Chem. 47 (1999), 3693) and PDA (Figure 10B).

[0050] The purpose of the analytical method is to detect the presence of the ten different individual procyanidins in fresh bilberry cells or freeze-dried cells. Detectable procyanidins are monomer, dimmers, trimers, tetramers, pentamers, hexamers, heptamers, octamers, nonamers and decamers.

[0051] The samples prepared from fresh bilberry cells, freeze-dried bilberry cells and bilberry cell extract were analyzed for procyanidin estimation using internally prepared procyanidin standards from cocoa beans by executing internal HPLC method on Empower 2.

Example 13. Scale-up of Bilberry Suspension Culture

[0052] A common problem in the use of plant cell cultures is obtaining consistent production of target products (Kim *et al., Biotechnol Prog.* 20(6) 1666, 2004). Therefore, a key for successful large-scale plant cell culture is to maintain stable productivity. A process to scale-up suspensions of bilberry cell cultures from 125 ml flasks to 250mls and then 500ml flasks was successfully conducted. The speed of the shakers was optimized for 500 ml flasks to give the same kind of growth and production numbers as in the 125 ml flasks. Three different shaker speeds were tested - 100, 110 and 120 RPM. The average PCV was 50~55% at seven days, which was about 2.5 times greater than the initial PCV level of 20% for all the treatments. However, the production yield (PY) was significantly high at 110 RPM when compared to 100 RPM with a P value of 0.005. Although the difference in PY was not significant between 110 RPM and 120 RPM, the

color in the 120 RPM flasks was slightly darker, leading to choose 110 RPM as preferred shaker speed for 500 ml flasks. Every seven days of culture, biomass, sugar concentration in medium, and polyphenol and/or procyanidin productivity, were measured.

[0053] Feasibility of scale up to 2.8L flasks is carried out, where shaking speed (rpm) and shaker stroke size is optimized. This successfully yields similar growth and production as in 125 ml and 500ml flasks.

Table 1

	VM1196	VM1204	VM1445	VM1222	VM1233	VM1491	VM1672
MS salts (g/L) (Phytotech Catalog # M524) (g/L)							4.33
Gamborg's B5 Salts (g/L) (Phytotech Catalog # G768)			3.1			3.1	
MS basal salts no nitrogen (g/L) (Phytotech Catalog # M531)	0.788						
B5 major salts modified[1] (ml/L) (20x) (EPS 000210 – Table 6)		50		50	50		
B5 minor salts (ml/L) (1000x) (EPS 0004 – Table 7)		1		1	1		
MS vitamins (mL/L) 1000X Stock Solution (Phytotech Catalog # M533)							
Ammonium Sulfate (mg/L)	3308						
Potassium Nitrate (mg/L)	808						
Nitsch and Nitsch (NN) vitamins (ml/L) 1000X Stock Solution (Phytotech Catalog # N608)	1						1
B5 Vitamins (ml/L) 1000x stock solution (Phytotech Catalog # G249)		1	1	1	1	1	
Ferrous Sulfate Chelate solution (100x) ml/L. (Phytotech Catalog # F318)	5	5	5	5	5		
Sucrose g/L	20	20	20	20	20	20	20

	VM1196	VM1204	VM1445	VM1222	VM1233	VM1491	VM1672
Agar g/L	7	7	7	7	7	7	7
Phytagel g/L							
Coconut water (Phytotech Catalog # C195)	100	100	100	100	100		
Casein Hydrolysate g/L						2	2
Polyvinylpyrrolidone PVP-10 (mg/L)					100		
1-Naphthalene acetic acid (NAA) (mg/L)	1	1	1		1		
2,4-D (mg/L)	0.1	0.1	0.1	1.0	0.1	1	1
Kinetin (mg/L)	0.5	0.5	0.5	0.5	0.5	1	1

Table 1 continued

	VM1443	VM1448	DC1152	VM1707	VM1516
MS salts (g/L) (Phytotech Catalog # M524) (g/L)			4.33	4.33	4.33
Gamborg's B5 Salts (g/L) (Phytotech Catalog # G768)					
MS basal salts no nitrogen (g/L) (Phytotech Catalog # M531)	0.788	0.788			
B5 major salts modified (ml/L) (20x) (EPS 000210 – Table 6)					
B5 minor salts (ml/L) (1000x) (EPS 0004 – Table 7)					
MS vitamins (mL/L) 1000X Stock Solution (Phytotech Catalog # M533)					
Ammonium Sulfate (mg/L)	1321	134			
Potassium Nitrate (mg/L)	808	808			
NN vitamins (ml/L) 1000X Stock Solution (Phytotech Catalog # N608)	1	1	1	1	1
B5 Vitamins (ml/L) 1000x stock solution (Phytotech Catalog # G249)					
Ferrous Sulfate Chelate solution (100x) ml/L. (Phytotech Catalog # F318)	5	5		5	5
Sucrose g/L	20	20	30	20	20
Agar g/L	7	7		7	7

	VM1443	VM1448	DC1152	VM1707	VM1516
Phytagel g/L			2.5		
Coconut water (Phytotech Catalog # C195)	100	100			100
Casein Hydrolysate g/L					
Polyvinylpyrrolidone PVP-10 (mg/L)					
NAA (mg/L)	1	1		1	1
2,4-D (mg/L)	0.1	0.1	1.5	0.1	0.1
Kinetin (mg/L)	0.5	0.5		0.5	0.5

Table 1 continued

	VM1570	VM1571	VM1572	VM1573	VM1727
MS salts (g/L) (Phytotech Catalog # M524) (g/L)					4.33
Gamborg's B5 Salts (g/L) (Phytotech Catalog # G768)	3.1	3.1	3.1	3.1	
MS basal salts no nitrogen (g/L) (Phytotech Catalog # M531)					
B5 major salts modified (ml/L) (20x) (EPS 000210 – Table 6)					
B5 minor salts (ml/L) (1000x) (EPS 0004 – Table 7)					
MS vitamins (mL/L) 1000X Stock Solution (Phytotech Catalog # M533)					
Ammonium Sulfate (mg/L)					
Potassium Nitrate (mg/L)					
NN vitamins (ml/L) 1000X Stock Solution (Phytotech Catalog # N608)					1
B5 Vitamins (ml/L) 1000x stock solution (Phytotech Catalog # G249)	1	1	1	1	
Ferrous Sulfate Chelate solution (100x) ml/L. (Phytotech Catalog # F318)					5
Sucrose g/L	20	20	20	20	20
Agar g/L	7	7	7	7	7
Phytagel g/L					
Coconut water (Phytotech Catalog # C195)				100	100
Casein Hydrolysate g/L	2.0	2.0	2.0	2.0	

	VM1570	VM1571	VM1572	VM1573	VM1727
Polyvinylpyrrolidone PVP-10 (mg/L)					
NAA (mg/L)		0.1	0.5		1
2,4-D (mg/L)	2.0	1.0	1.0	1.0	0.5
Kinetin (mg/L)	1.0	1.0	1.0	1.0	0.5

Table 2 Composition of media for suspension cultures

Components	VM1799	VM1831	VM1901	VM1933	VM3071	VM3072	VM3103
MS salts g/L (Phytotech Catalog # M524) (g/L)	4.33	4.33	4.33	4.33	4.33	4.33	4.33
NN vitamins ml/L 1000X Stock Solution (Phytotech Catalog # N608)	1	1	1	1	1	1	1
Coconut water ml/L (Phytotech Catalog # C195)		100	100	100	100	100	100
Sucrose g/L	30	20	20	30	30	30	30
Ferrous Sulfate Chelate solution (100x) ml/L. (Phytotech Catalog # F318)		5		5	5	5	5
IAA (mg/L)							
NAA(mg/L)		1.0	1.0	1.0	1.0		
IBA (mg/L)					0.1	1.0	2.0
2,4-D (mg/L)	1.5	0.1	0.1	0.1			
Kinetin (mg/L)	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 2 continued

Components	VM3014	VM3110	VM3111	DC1151
MS salts g/L (Phytotech Catalog # M524) (g/L)	4.33	4.33	4.33	4.3
NN vitamins ml/L 1000X Stock Solution (Phytotech Catalog # N608)	1	1	1	1
Coconut water ml/L (Phytotech Catalog # C195)	100	100	100	
Sucrose g/L	30	20	20	30
Ferrous Sulfate Chelate solution (100x) ml/L. (Phytotech Catalog # F318)	5	5		
IAA (mg/L)		1.0	2.0	
NAA(mg/L)				
IBA (mg/L)	3.0			

Components	VM3014	VM3110	VM3111	DC1151
2,4-D (mg/L)				1.5
Kinetin (mg/L)	0.5	0.5	0.5	

[0054] The present disclosure may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the disclosure is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

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In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

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CLAIMS

What is claimed is:

1. A cell culture, comprising:

a plurality of friable *Vaccinium myrtillus* cells growing in a suspension cell culture, the cells being derived from one or more of:

a hypocotyl, a cotyledon, a leaf section, a stem section, or a root section of a seedling; or

a berry, a stem section including a node or an internode, or a leaf section of a mature plant,

wherein the plurality of *Vaccinium myrtillus* cells are selected to be capable of obtaining a packed cell volume of at least 55% in 7 days of growth in the suspension cell culture, and

wherein at least 5% of a dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins.

2. The cell culture of claim 1, wherein at least 10%, 15%, or 20% of the dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of polyphenols.

3. The cell culture of claim 1 or 2, wherein at least 7.5% of the dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins.

4. The cell culture of any one of claims 1 to 3, wherein at least 10% of the dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins.

5. The cell culture of any one of claims 1 to 4, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.5% anthocyanin.

6. The cell culture of any one of claims 1 to 5, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.1% anthocyanin.

7. The cell culture of any one of claims 1 to 6, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.01% anthocyanin.

8. The cell culture of any one of claims 1 to 7, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.001% anthocyanin.

9. The cell culture of any one of claims 1 to 8, wherein the procyanidins comprise dimers, trimers, tetramers, pentamers, hexamers, heptamers, octamers, nonamers and decamers.

10. The cell culture of any one of claims 1 to 9, wherein the production of polyphenols in the plurality of *Vaccinium myrtillus* cells is greater than 3 grams per liter of packed cells.

11. The cell culture of any one of claims 1 to 10, wherein the production of polyphenols in the plurality of *Vaccinium myrtillus* cells is greater than 7 grams per liter of packed cells.

12. The cell culture of any one of claims 1 to 11, wherein the plurality of *Vaccinium myrtillus* cells are selected to be capable of doubling in density within 7 days or less of growth in the suspension cell culture.

13. The cell culture of any one of claims 1 to 12, wherein the liquid medium comprises a carbohydrate source, major salts, minor salts, and one or more hormones selected from the group consisting of an auxin and a cytokinin

14. A method of increasing growth of *Vaccinium myrtillus* cells in suspension cell culture, the method comprising:

providing a suspension cell culture of *Vaccinium myrtillus* cells;

subculturing the cells in a liquid medium in suspension cell subculture; and

selecting suspension cell subcultures having greater than 45% packed cell volume (PCV) after 7 days of growth, at least 5% of a dry mass of the *Vaccinium myrtillus* cells in the selected suspension cell cultures being comprised of procyanidins, and less than 0.5% of the dry mass of the *Vaccinium myrtillus* cells in the selected suspension cell cultures being comprised of anthocyanin.

15. The method of claim 14, wherein providing a suspension cell culture of *Vaccinium myrtillus* cells comprises:

producing a cell callus of *Vaccinium myrtillus* cells derived from one or more *Vaccinium myrtillus* plant parts;

introducing one or more cells derived from the callus into a liquid medium;

agitating the one or more cells in the liquid medium;

replacing the liquid medium with a fresh liquid medium or transferring the cells to fresh a fresh liquid medium to establish the suspension cell culture of *Vaccinium myrtillus*.

16. The method of claim 14 or 15, wherein about 10% of the dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins.

17. The method of any one of claims 14 to 16, wherein the plurality of *Vaccinium myrtillus* cells contain less that about 0.1 % anthocyanin or the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.1% anthocyanin.

18. The method of any one of claims 14 to 17, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.01% anthocyanin.

19. The method of any one of claims 14 to 18, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.001% anthocyanin or the plurality of *Vaccinium myrtillus* cells are substantially free of anthocyanin.

20. The method of any one of claims 14 to 19, wherein the liquid medium comprises a carbohydrate source, major salts, minor salts, and one or more hormones selected from the group consisting of an auxin and a cytokinin.

21. A method of extracting polyphenols from *Vaccinium myrtillus* cells in culture, the method comprising:

selecting a plurality of *Vaccinium myrtillus* cells adapted to grow in suspension culture, the cells being derived from one or more of:

a hypocotyl, a cotyledon, a leaf section, a stem section, or a root section of a seedling; or

a berry, a stem section including a node or an internode, or a leaf section of a mature plant; and

extracting polyphenols from the cells using a solvent,

wherein at least 10% of a dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of polyphenols and at least 5% of a dry mass of the plurality of *Vaccinium myrtillus* cells is comprised of procyanidins.

22. The method of claim 21, wherein the dry mass of the plurality of *Vaccinium myrtillus* cells comprises less than 0.5% anthocyanin.

23. The method of claim 22, further comprising selecting *Vaccinium myrtillus* cells having at least 10% of the dry mass of the plurality of *Vaccinium myrtillus* cells comprised of polyphenols, at least 5% of the dry mass of the *Vaccinium myrtillus* cells comprised of procyanidins, and less than 0.5% of the dry mass of the *Vaccinium myrtillus* cells comprised of anthocyanin.

24. The method of claim 23, further comprising:

producing a cell callus of *Vaccinium myrtillus* cells derived from one or more *Vaccinium myrtillus* plant parts;

introducing one or more cells derived from the callus into a liquid medium;

agitating the one or more cells in the liquid medium;

replacing the liquid medium with a fresh liquid medium or transferring the cells to fresh a fresh liquid medium to establish a suspension cell culture of *Vaccinium myrtillus*;

subculturing the suspension cell culture of *Vaccinium myrtillus* cells; and

selecting suspension cell subcultures having at least 10% of the dry mass of the plurality of *Vaccinium myrtillus* cells comprised of polyphenols, at least 5% of a dry mass of the *Vaccinium myrtillus* cells in the selected suspension cell cultures comprised of procyanidins, and less than 0.5% of the dry mass of the *Vaccinium myrtillus* cells comprised of anthocyanin.

25. The method of claim 24, further comprising selecting suspension cell subcultures having greater than 45% packed cell volume (PCV) after 7 days of growth.

26. A polyphenol extracted by the method of any one of claims 21 to 25.

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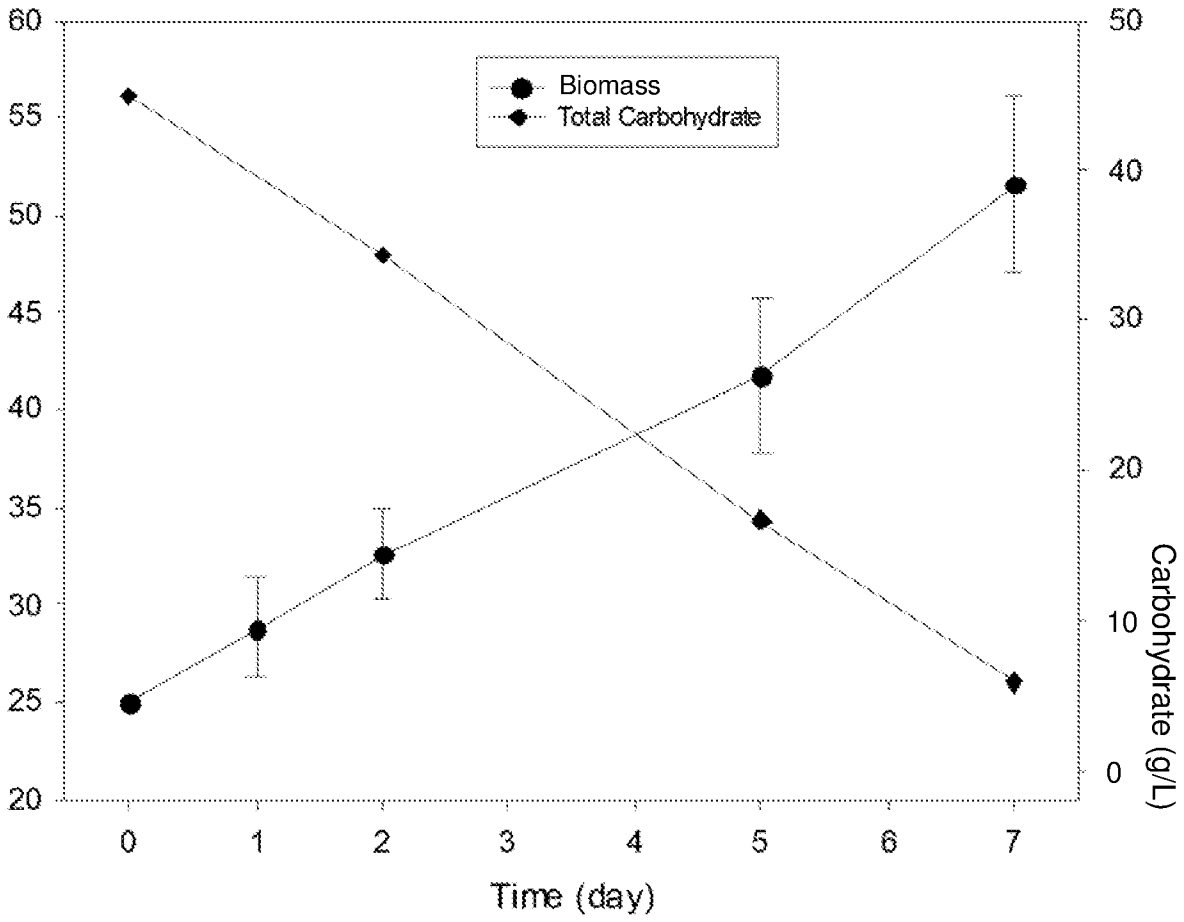

Figure 1

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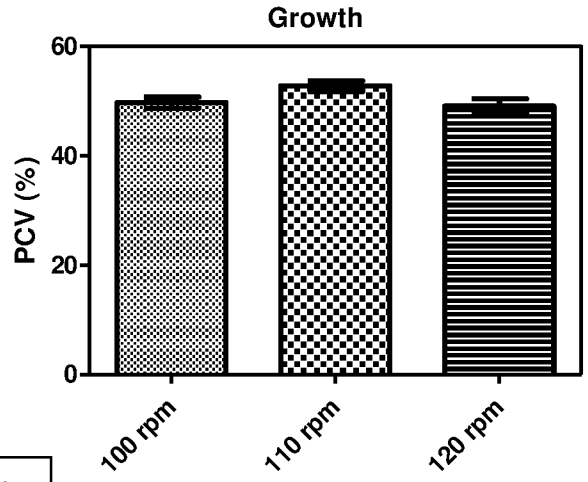

Figure 2a

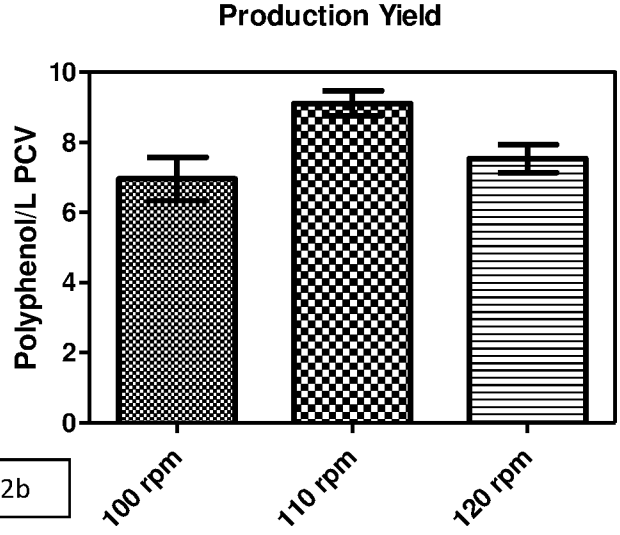

Figure 2b

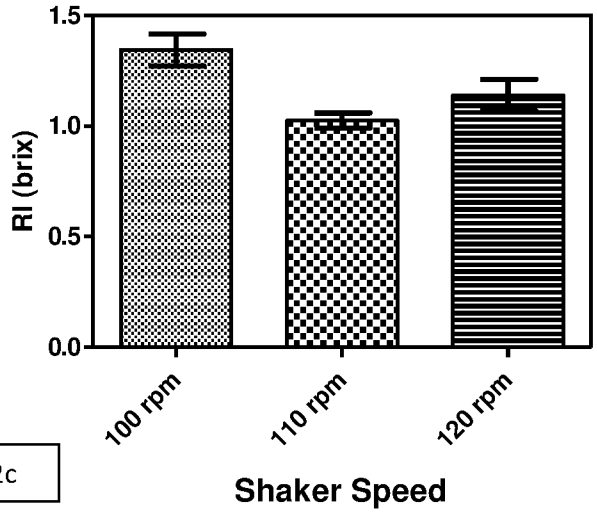

Figure 2c

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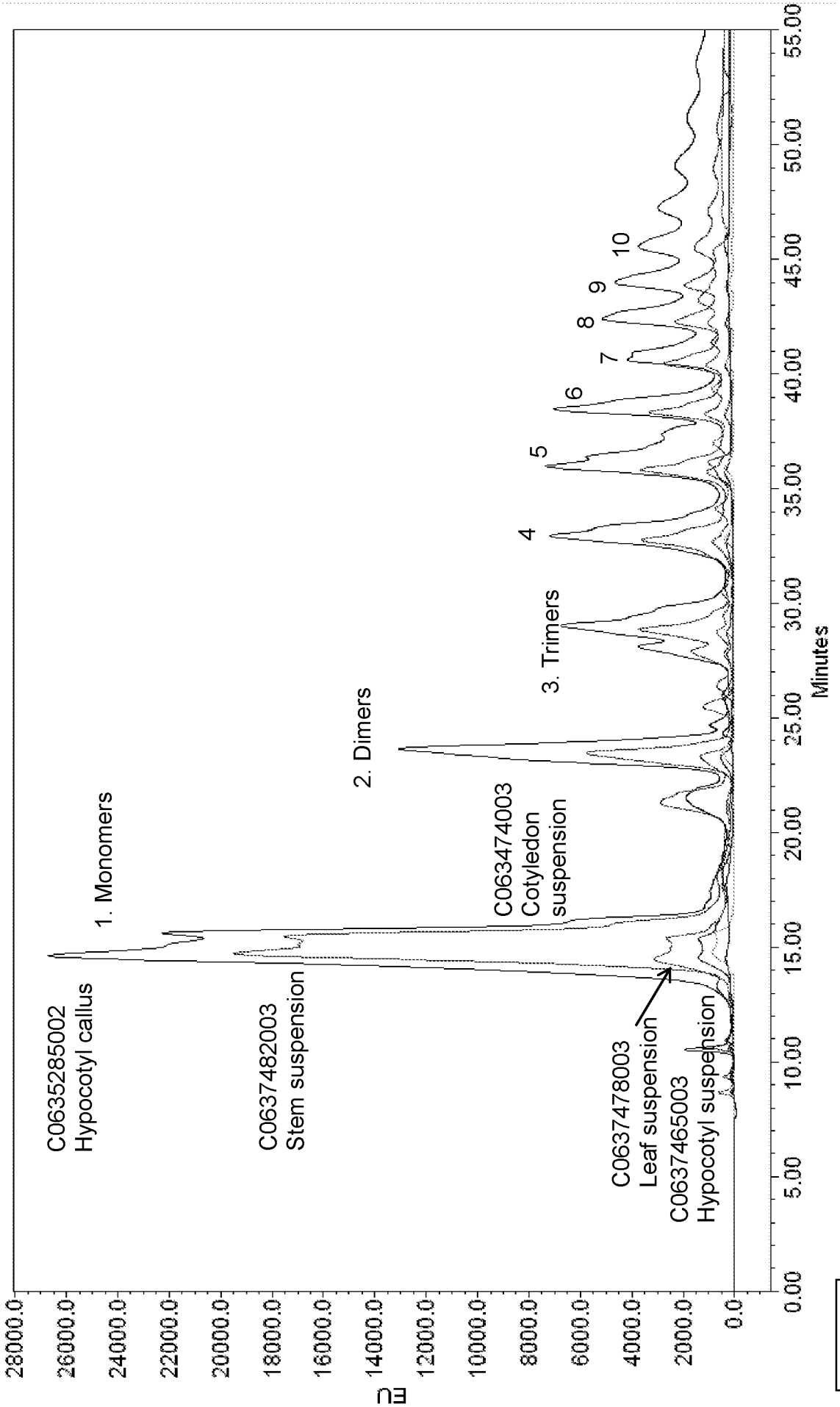

Figure 3

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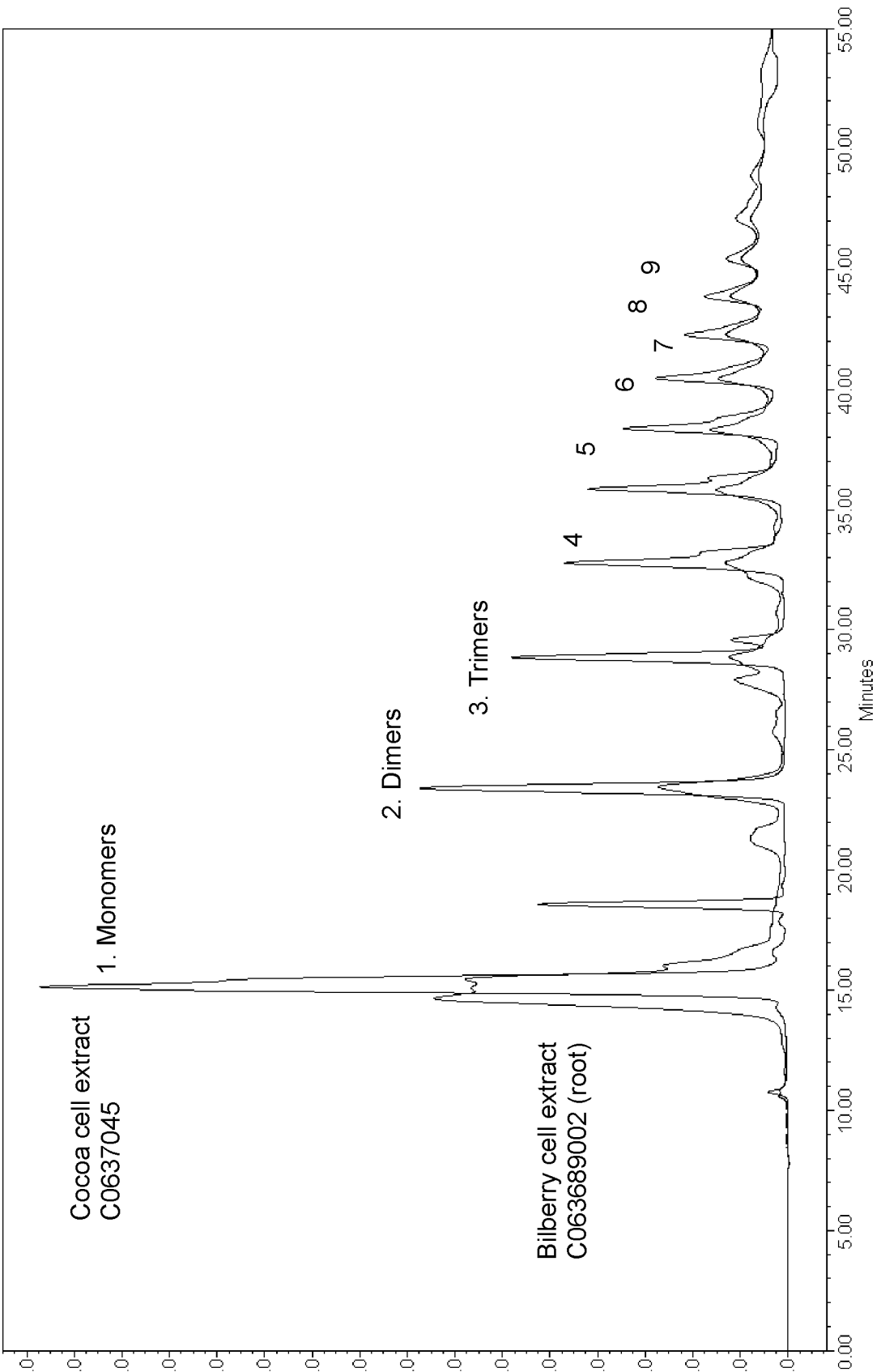

Figure 4

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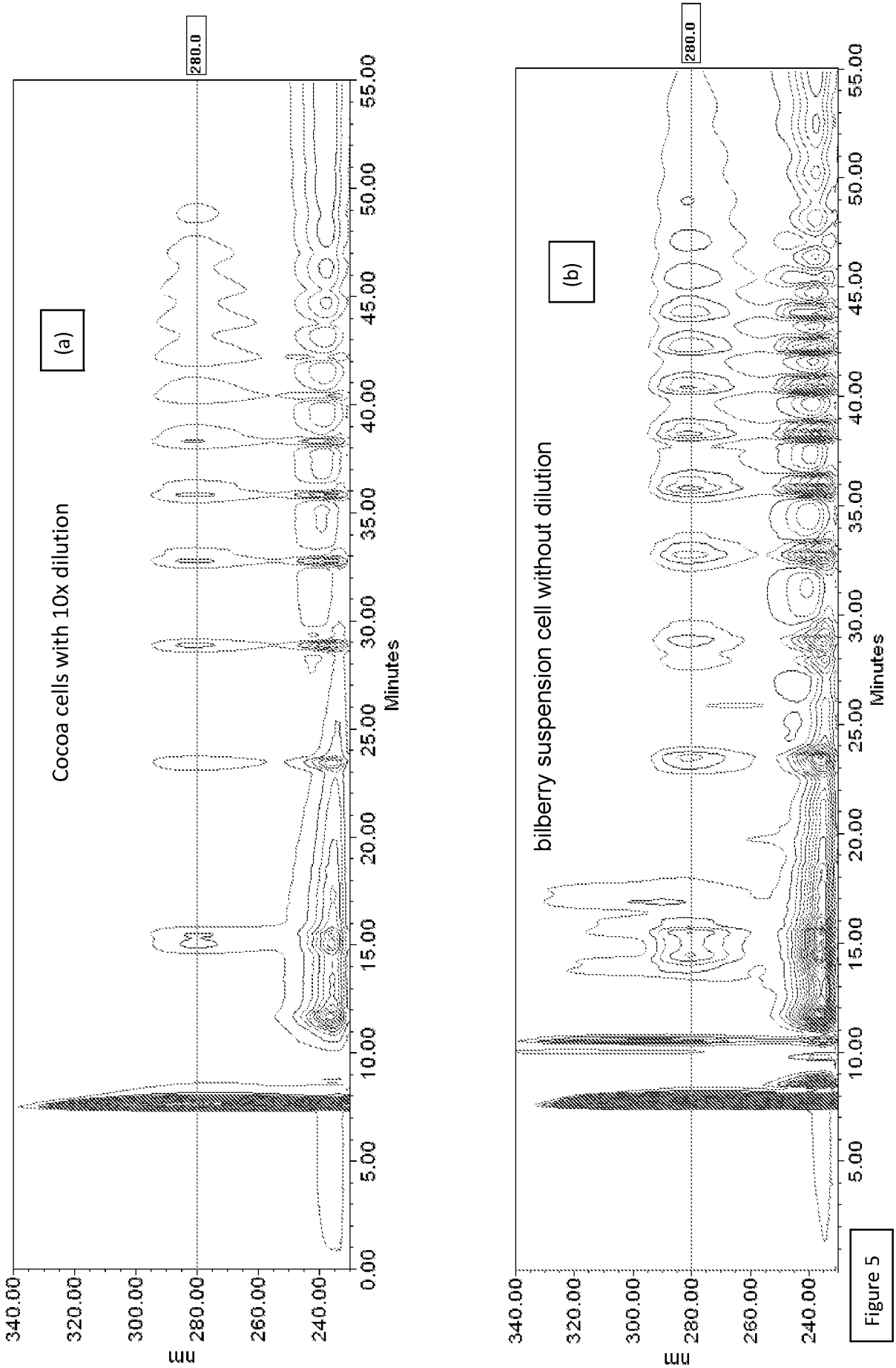

Figure 5