

PLANT-DERIVED SERUM REPLACEMENT FOR CELLULAR AGRICULTURE

TECHNICAL FIELD

- 5 The present invention relates to a plant-derived serum (PDS) replacement for substituting animal-derived serum that is used as supplement in cell culture media, a cell culture medium comprising such PDS replacement, and application of the PDS replacement in cellular agriculture.

BACKGROUND OF THE INVENTION

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Cultivated meat (CM) or cellular agriculture is an emerging field in the production of agricultural products such as meat from lab-cultured cells. CM technologies provide a new food resource to buffer against unforeseen disruptions in traditional food supply chains, and avoids issues associated with animal farming such as cruelty, greenhouse gas emissions, inefficient energy
15 conversion, and high land requirements.

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Hence, CM is particularly important in land scarce urban communities which have limited domestic agricultural output and is viewed as a viable alternative to traditional sources of meat to realize Singapore's "30-by-30" goal, i.e. to produce 30 % of nutritional needs locally and
20 sustainably by year 2030.

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Currently, there are a handful of Singapore-based CM companies and more than 40 such companies globally. Recently, Singapore became the first country to approve a cultured chicken nugget CM product developed by Eat Just for sale nationwide. However, the relative high price of
25 CM products is a major obstacle to widespread adoption of these CM products.

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One major cost factor lies in the ubiquitous use of animal-derived serum such as fetal calf serum (FCS) in cell culture media used to cultivate meat in the laboratory. These animal-derived serum are thought to play an important role in promoting *in vitro* cell growth. However, besides being
30 costly, the animal-derived serum also poses other scientific and ethical concerns.

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One particular concern is N-glycolylneuraminic acid (Neu5Gc) contamination of CM products. Neu5Gc is a sialic acid that humans are deficient in (Padler-Karavani, Vered, et al. "Diversity in specificity, abundance, and composition of anti-Neu5Gc antibodies in normal humans: potential implications for disease." *Glycobiology* 18.10 (2008): 818-830). Neu5Gc is typically metabolically incorporated into tissues from the diet and is the first example of a “xeno-autoantigen” (Dhar, Chirag, Aniruddha Sasmal, and Ajit Varki. "From “serum sickness” to “xenosialitis”: Past, present, and future significance of the non-human sialic acid Neu5Gc." *Frontiers in immunology* 10 (2019): 807). Neu5Gc concentrations are high in red meats such as beef, pork, and lamb. Plants and poultry do not contain Neu5Gc while fish have been documented to contain low to trace amounts of Neu5Gc (Li, Hongwei, and Xingdan Fan. "Quantitative analysis of sialic acids in Chinese conventional foods by HPLC-FLD." *Open Journal of Preventive Medicine* 2014 (2014)). However, any mammalian, fish or crustacean cell line grown in FCS will likely sequester Neu5Gc from the culture media and this phenomenon is well recognized (Martin, Maria J., et al. "Human embryonic stem cells express an immunogenic nonhuman sialic acid." *Nature medicine* 11.2 (2005): 228-232). The lysosomal sialic acid transporter has been reported as the mechanism responsible for the metabolic incorporation of free Neu5Gc (Banda, Kalyan, et al. "Metabolism of vertebrate amino sugars with N-glycolyl groups: mechanisms underlying gastrointestinal incorporation of the non-human sialic acid xeno-autoantigen N-glycolylneuraminic acid." *Journal of Biological Chemistry* 287.34 (2012): 28852-28864).

Concerns with Neu5Gc are not new. More than a decade ago, fears with the use of FCS in stem cell cultures lead rapidly to the development of “xeno-free” culture media and protocols for regenerative medicine applications (Li, Hongwei, and Xingdan Fan. "Quantitative analysis of sialic acids in Chinese conventional foods by HPLC-FLD." *Open Journal of Preventive Medicine* 2014 (2014)). Chronic inflammation or “xenosialitis” brought about by metabolic incorporation of Neu5Gc has important implications in human health and disease (Ma, Fang, et al. "A mouse model for dietary xenosialitis: antibodies to xenoglycan can reduce fertility." *Journal of Biological Chemistry* 291.35 (2016): 18222-18231). There is also substantial evidence to demonstrate that antibodies against the red meat-derived Neu5Gc exacerbate cancer in “human-like” mice (Samraj, Annie N., et al. "A red meat-derived glycan promotes inflammation and cancer progression." *Proceedings of the National Academy of Sciences* 112.2 (2015): 542-547). Human anti-Neu5Gc

antibodies and red meat have also been independently proposed to increase cancer risk (*Pearce, Oliver MT, and Heinz Läubli. "Sialic acids in cancer biology and immunity." Glycobiology 26.2 (2016): 111-128.*

- 5 The possibility remains that animal-derived serum such as FCS will still be used to manufacture CM products, albeit in far smaller quantities as the technology advances. Nevertheless, the first CM products rolled off the shelf are likely to be tainted with FCS. Indeed, Eat Just's culture chicken nuggets are reportedly grown or have been exposed to FCS (*Waltz, Emily. "Club-goers take first bites of lab-made chicken." Nature Biotechnology (2021)).*

10 Neu5Gc-incorporation phenomena are practically significant in the consumption of CM products because humans can have high levels of circulating anti-Neu5Gc antibodies (*Padler-Karavani, Vered, et al. "Diversity in specificity, abundance, and composition of anti-Neu5Gc antibodies in normal humans: potential implications for disease." Glycobiology 18.10 (2008): 818-830.*

- 15 Therefore, use of animal-derived serum like FCS to manufacture CM products is of concern and consumers should be properly evaluated for Neu5Gc bioaccumulation.

Many have sought to substitute animal-derived serum such as FCS with other supplements in serum-free culture media. In this regard, serum-free culture media and continuous cell lines
20 adapted to serum-free culture media have been extensively studied, with most common serum replacements including the following: hormones, growth factors such as fibroblast growth factor (FGF), insulin-transferrin-selenium (ITS), adhesion factors such as fibronectin, bovine serum albumin, oleic and linoleic acid (1-3).

- 25 However, the switch to serum-free culture media demands a time-consuming literature survey and search for appropriate medium formulations from suppliers.

Therefore, there is a need in the art for a low-cost serum replacement for substituting animal-derived serum currently used in cell culture media. Such serum replacement should ideally also be
30 easily prepared, scalable, and comprises most, if not all, of the components necessary for cellular proliferation and survival. The development of such low-cost serum replacement will enable cost-

effective large-scale production of CM products, making it feasible to commercialize additional CM products at near price parity with traditional meat.

SUMMARY OF THE INVENTION

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The above and other problems in the art are solved and an advance in the art is made in accordance with this invention.

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A first advantage of a serum replacement in accordance with this invention is that the serum replacement is plant-derived and hence free of infectious agents such as prions. A second advantage of a serum replacement in accordance with this invention is that the serum replacement is easy to prepare and upscale. A third advantage of a serum replacement in accordance with this invention is that the serum replacement comprises most, if not all, the components necessary for cellular proliferation and survival.

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In a first aspect, a PDS replacement comprising one or more aqueous extract of a legume pulse or pulp thereof is provided.

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In an embodiment, the PDS replacement comprises an aqueous extract of Okara.

In some embodiments, the PDS replacement comprises one or more aqueous extract of legume pulse selected from a group consisting of soybean, chickpea, mung bean, lentil and quinoa.

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The PDS replacement of the invention comprises albumin and/or transferrin that may be enriched.

In a second aspect, a cell culture medium comprising a PDS replacement of the present invention is provided. The cell culture medium may not contain any animal-derived supplements.

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In a third aspect, the invention relates to use of the PDS replacement and the cell culture medium of the present invention to cultivate animal cells, particularly in manufacture of CM products.

In a preferred embodiment, the invention relates to use of a PDS replacement comprising an aqueous extract of Okara or a cell culture medium containing the aqueous extract of Okara to culture fish cells.

5 In a fourth aspect, a method of preparing a PDS replacement is provided. The method comprises adding water to a legume pulse or pulp thereof to obtain a mixture; blending the mixture; and isolating a solution from the blended mixture to obtain a PDS replacement comprising one or more aqueous extract of the legume pulse or pulp thereof.

10 In an embodiment, the step of adding water to a legume pulse or pulp thereof to obtain a mixture comprises adding water to Okara.

In some embodiments, the water is added to the legume pulse selected from a group consisting of soybean, chickpea, mung bean, lentil and quinoa.

15 Preferably, the water is added to the legume pulse or pulp thereof in a 4:1 mass ratio.

In some embodiments, the solution is isolated from the blended mixture by filtering and/or centrifuging.

20 The method may further comprise heating the legume pulse or pulp thereof, prior to mixing with water, to reduce antinutrient content.

The method may also further comprise subjecting the solution to a bioprocess treatment such as enzymatic digestion using a protease (for example, subtilisin) or fermentation.

25 In some embodiments, the method may further comprise enriching an albumin and/or transferrin content of the solution.

30 **BRIEF DESCRIPTION OF THE DRAWINGS**

FIG 1 is a schematic that outlines the steps for preparing a PDS replacement in accordance with an embodiment of the present invention and application to cell culture.

FIG. 2 shows a SDS-PAGE gel analysis of a Okara-derived PDS, indicating presence of plant transferrin and albumin.

FIG. 3 shows the results for substituting FCS with a Okara-derived PDS replacement in a cell culture medium for culturing a piscine cell line, BF-2.

FIG. 4 shows results for culturing grouper cells in L-15 basal medium supplemented with a Okara-derived PDS and influence of ITS supplement.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides essentially a plant-derived serum (PDS) replacement with a high nutritional index that is supportive of *in vitro* animal cell growth while reducing or eliminating the need to supplement cell culture media with an animal-derived serum/supplement.

In the following description, a number of terms are used extensively. Hence, to provide a clear understanding of the invention, the following definitions, including the scope of certain terms, are provided.

“Cell culture” refers to maintenance of cells in an artificial, *in vitro* environment, under conditions favoring continued viability, growth or proliferation and/or differentiation.

“Cell culture medium” or “cell culture media” (plural) refers to a nutritive solution for cell culture. “Culture medium” may be used interchangeably with “cell culture medium”.

“Pulse” refers to a seed of a legume.

“Basal medium” or “basal media” (plural) refers to a solution comprising a number of components

for cell culture including one or more of the following: amino acids, vitamins, organic salts, inorganic salts, trace elements, buffering salts, sugar, ATP and the like. Examples of basal media include but are not limited to Dulbecco's Modified Eagle Medium (DMEM), Leibovitz's L-15 (L-15), RPMI 1640 Medium, and the like.

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"Supplement" typically includes serums, growth factors, hormones, cytokines and the like that are added to a basal medium to enhance cell culture.

10 "Serum" refers to the fluid component of blood, with "animal-derived serum" being a serum obtained from an animal. Examples of animal-derived serum include but are not limited to FCS, horse serum, fetal bovine serum and the like.

15 "Aqueous extract" refers to a composition formed by treating a material mechanically (e.g. blending, grinding, cutting, pressurizing, etc) and/or chemically (e.g. distillation) in an aqueous environment.

The PDS replacement of the present invention is obtained from an aqueous extraction of a legume pulse including soybean, chickpea, mung bean, lentil and quinoa.

20 In some embodiments, a pulp that is obtained from processing the legume pulse is used for the aqueous extraction. For instance, as would be exemplified in the Examples below, Okara or tofu dreg that is the pulp consisting of insoluble parts of the soybean that remain after pureed soybeans are filtered during production of soy milk or tofu, may be used.

25 In some embodiments, the PDS replacement may comprise more than one aqueous extract of a legume pulse or pulp thereof.

30 In an embodiment, the aqueous extraction is performed by adding water to the legume pulse or pulp thereof, then blending the mixture and isolating a solution from the blended mixture.

Preferably, the water is added to the legume pulse or pulp thereof in a 4:1 mass ratio to obtain the

mixture that is blended for 5 minutes. The blended mixture is then filtered with a strainer to remove large solids, and the filtrate is centrifuged with the supernatant further filtered through a filter membrane (0.2 micron pore size) to obtain the PDS replacement.

- 5 In some embodiments, prior to adding water, the legume pulse or pulp thereof is heated to reduce antinutrient content.

In some embodiments, the PDS replacement is further subjected to a bioprocess treatment to modify a nutritional content of the PDS replacement. For instance, the PDS replacement may be
10 subjected to enzymatic digestion using a protease such as subtilisin to vary the amino acid content, or undergo fermentation to alter the sugar content.

The PDS replacement is rich in plant albumin and transferrin which make the serum replacement suitable for cell culture. The albumin and transferrin content may be further enriched by processes
15 known by the skilled person in the art, for instance, via sequential alkalization and acidification extraction.

The PDS replacement may be used on its own for cell culture, or used to supplement a basal medium for cell culture. Where used to supplement a basal medium, the cell culture medium needs
20 not comprise an animal-derived serum, or only requires a reduced amount of an animal-derived serum such as 7.5 %, 5%, 2.5% or preferably 1% animal-derived serum by weight.

The PDS replacement or cell culture medium comprising the PDS of the present invention may be used to culture animal cells.

25 The above is exemplified in the Examples below which are not to be seen as limiting to the scope of the invention. One skilled in the art will recognize that the examples set out below are not an exhaustive list of the embodiments of this invention. FIG 1 outlines the steps for preparing a PDS replacement in accordance with an embodiment of the present invention and application to cell
30 culture.

EXAMPLES

Example 1: Preparation of PDS from Okara

5 The preparation of a PDS replacement from Okara is exemplified. The inventors are cognizant that serum substitutes need to be cost-effective. In this regard, valorized plant by-products such as Okara has the potential to be a cheap and readily available source for PDS while reducing carbon footprint. It is estimated that the cost of Okara-derived PDS may be less than S\$1/liter which is about 1000-fold cheaper than most commercially available pharmaceutical grade FCS products.

10 Briefly, Okara is collected fresh from soy milk or tofu production plants and chilled quickly to prevent spoilage. The Okara is then subjected to heating steps to reduce antinutrient (e.g. phytate) content. The heat-treated Okara is then mixed with MilliQ water and transferred into a blender. After blending the Okara mixture for 5 mins, the mixture is filtered with a strainer. The filtrate is
15 then centrifuged at 350G twice and the supernatant is filtered through a filtration membrane (0.2 micron pore size) to obtain the Okara-derived PDS (PDS-01).

The volume of water used per unit mass raw material, filtration, extraction and centrifugation conditions are key to preserving a high nutrient profile in the Okara-derived PDS. In this example,
20 preferably 100g of Okara is mixed with 400 ml MilliQ water, i.e. 1:4 mass ratio.

The Okara-derived PDS may be subjected to a bioprocess treatment such as enzymatic digestion using proteases like Subtilisin, or fermentation to enhance the macronutrient profile.

25 Example 2: Nutrition analysis of Okara-derived PDS

The macronutrient including protein, lipid, carbohydrate, and mineral profile of PDS-1 has been analyzed, see Table 1, and is suggestive of a high nutritional index supportive of *in vitro* cell growth.

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Table 1: Macronutrient analysis of PDS-1.

Sample ID				Cell culture serum	---
Sampling date/time				14-Dec-2020	----
				00:00	
Method	Test Parameter	Unit	LOD	SF2044190-001	-----
FC23	Carbohydrate (including TDF)	g/100g	0.1	0.3	----
FC118	Protein (Nx6.25)	g/100g	0.1	0.2	----
FC91	Glucose	% (w/w)	0.1	Not Detected	----
FC91	Fructose	% (w/w)	0.1	Not Detected	----
FC91	Lactose	% (w/w)	0.1	Not Detected	----
FC91	Maltose	% (w/w)	0.1	Not Detected	----
FC91	Sucrose	% (w/w)	0.1	Not Detected	----
FC91	Total Sugars (Glucose, Fructose, Lactose, Maltose & Sucrose)	% (w/w)	-	Not Detected	----
FC25	Calcium as Ca	ppm	0.5	36.5	----
FC25	Magnesium as Mg	ppm	0.2	50.6	----
FC25	Phosphorus as P	ppm	0.2	168	----
FC25	Potassium as K	ppm	0.5	561	----
FC25	Selenium as Se	ppm	0.20	<0.20	----
FC25	Sodium as Na	ppm	1	3.79	----
FC25	Zinc as Zn	ppm	0.20	2.88	----
AOCS_Ce_1h_05FULL	C14:0 (Myristic Acid)	% wt	0.01	0.1	----
AOCS_Ce_1h_05FULL	C16:0 (Palmitic Acid)	% wt	0.01	16.8	----
AOCS_Ce_1h_05FULL	C16:1 (Palmitoleic Acid)	% wt	0.01	0.1	----
AOCS_Ce_1h_05FULL	C17:0 (Heptadecanoic Acid)	% wt	0.01	0.2	----
AOCS_Ce_1h_05FULL	C18:0 (Stearic Acid)	% wt	0.01	12.3	----
AOCS_Ce_1h_05FULL	C18:1 (Oleic Acid)	% wt	0.01	19.5	----
AOCS_Ce_1h_05FULL	C18:2 (Linoleic Acid)	% wt	0.01	44.6	----
AOCS_Ce_1h_05FULL	C18:3 (Linolenic Acid)	% wt	0.01	5.4	----
AOCS_Ce_1h_05FULL	C20:0 (Arachidic Acid)	% wt	0.01	0.5	----
AOCS_Ce_1h_05FULL	C22:0 (Behenic Acid)	% wt	0.01	0.3	----
AOCS_Ce_1h_05FULL	C24:0 (Lignoceric Acid)	% wt	0.01	0.2	----
AOCS_Ce_1h_05FULL	Fat - Total	g/100g	0.05	0.2	----

The Okara-derived PDS, i.e. PDS-1, contains amino acids and high concentrations of essential fatty acids such as linolenic and linoleic acid which makes up almost 50% of the fatty acid. Fatty acids are needed by animal cells for long-term energy storage. In addition, fatty acids are also precursors of other molecules (eg. prostaglandins and glycolipids) and play an important structural role. Salts and trace elements are also present.

Typically, the elimination of FCS with its complement of natural lipid carriers, poses significant problems to media supplementation because animal cells cannot synthesize fatty acids of the n-3 or n-6 type. These must be provided for nourishment and are considered essential fatty acids.

Based on Table 1, the inventors believe PDS-01 to be deficient in sulphur containing amino acids (e.g. methionine, serine).

As shown in FIG. 2 which shows a SDS-PAGE gel analysis of PDS-01 (for performing the SDS-PAGE gel electrophoresis, see *Kusumah, Jennifer, Luis M. Real Hernandez, and Elvira Gonzalez de Mejia. "Antioxidant potential of mung bean (Vigna radiata) albumin peptides produced by enzymatic hydrolysis analyzed by biochemical and in silico methods." Foods 9.9 (2020): 1241.*)

that indicates presence of plant transferrin and albumin that are important in serum-free culture media.

The macronutrient profile of PDS-1 is compared against a basal medium (e.g. DMEM) and FCS in Table 2. Compared with basal media and FCS, PDS-01 carbohydrate concentration is at 3 ppt.

The major carbohydrate types in PDS-01 are believed to be arabinose, galactose, xylose, and galacturonic acid. In terms of amino acids, PDS-01 contains a total of 2 ppt of total protein.

The inventors postulate that bioprocess treatment such as protein digestion with subtilisin will liberate free amino acids.

Table 2: Macronutrient comparison between PDS-1, basal medium and FCS.

Media	Carbohydrate (ppt)	Carbohydrate (type)	Amino Acid (ppt)	Amino Acid (type)	Linoleic & Linolenic acids essential fatty acids (% total fatty acids)
PDS-01	3.0	Arabinose, galactose, xylose, galacturonic acid	2.0	Deficient in sulphur containing amino acids (e.g. methionine, serine)	Approx. 50% of total fatty acids
Basal Media (e.g. DMEM)	1.0 to 4.5	Mainly glucose or galactose (L15)	0.6 to 3.3	All essential amino acids	Typically provided by FCS
Fetal Calf Serum (FCS)	Typically provided by basal media				Approx. 12% of total fatty acids

Example 3: Application of Okara-derived PDS

The inventors have used the Okara-derived PDS, PDS-1, to sustain the growth of two mammalian cell lines (MDCK and HEK-293), a piscine cell line (BF-2, Blue Gill), and cultivated meat piscine cell lines from Grouper, Snapper, and Unagi (*Anguilla japonica*) for over 3 months *in vitro* and for over 45 population doublings with high cell viability even after replacing 90% of FCS with PDS-01. Other legume-derived PDS which are supportive of *in vitro* cell growth may be derived from chickpeas, mung beans, lentils, soybean, and quinoa and application of legume-derived PDS in different ratios and proportions lead to different cell growth outcomes.

The Okara-derived PDS, PDS-1 is mixed with a basal media such as DMEM in varying proportions and used to culture the piscine cell line, BF-2, see results in FIG. 3. The control refers to DMEM supplemented with 10% by weight of FCS. SR refers to plant-derived serum replacement. As an example, the 25% SR culture medium refers to DMEM supplemented with 2.5% PDS-1 and 7.5% FCS. FIG. 3A shows the normalized cell yield relative to the control. It can be seen that high yield, comparable to the control, can be achieved even at 90% SR, i.e. only 1% FCS in the culture media.

FIG 3B shows a phase contrast image of BF-2 cells cultured for 4 weeks in 90% SR culture medium and FIG 3C shows a phase contrast image of BF-2 cells cultured for 4 weeks in the control culture medium. Both images show high cell viability.

The inventors also discovered that the okara source is very important as not all okara material obtained from suppliers performed equally well (results not shown).

Further, the inventors show that *bona fide* piscine cultivated meat cell lines from grouper species can be adapted to low serum and serum-free conditions with Okara-derived PDS supplementation. Cells grown in basal media supplemented with Okara-derived PDS show superior growth compared to low-serum controls. Supplementing with Insulin-Transferrin-Selenium (ITS) does not appear to be necessary.

The above is demonstrated in FIG. 4 which shows phase contrast images of grouper cells in supplemented L-15 basal medium at Day 3, Passage 10. FIG. 4A and 4C, represent low serum conditions (i.e. 1.5 % fetal bovine serum), with the cell culture medium in 4C further supplemented with ITS. FIG. 4B and 4D, represent serum-free conditions where PDS-01 is used as serum substitute (i.e. 8.5 % PDS-01), with the cell culture medium in 4D further supplemented with ITS.

Given the promising composition of Okara-derived PDS, it may be possible to do away with many components of the basal media (for example, DMEM, L-15) or at the very least reduce basal media proportions in the final culture media formulation. This will further contribute to cost reduction.

The inventors postulate that this may potentially be achieved via bioprocess treatment such as fermentation and/or enzymatic digestion to liberate peptides, amino acids, and simple sugars to give a complete media substitute that would require just the addition of a sugar source such as glucose or galactose, an appropriate buffer systems (for example, phosphate or bicarbonate), and sterile water.

Comparison with competing technologies

Table 3 summarizes some of the benefits and drawbacks of different competing solutions versus PDS of the present invention.

First, for PDS-01, the raw material and final product can be of very low cost, which makes it ideal for large scale manufacture of CM products which will require large quantities of culture media. PDS-01 is also of valorised origin and from a low-cost food product that is widely consumed in Singapore and Asia. Therefore, availability will not be an issue. It is possible that the consistency of PDS-01 raw material may vary depending on the processes used by different food companies from which Okara is obtained. However, this can be mitigated by building close relationships with a few key food companies and working closely with the companies to standardize processes to reduce batch variation. As with many other food products, there may be presence of allergens in PDS. Some people may be allergic to legumes for instance, but the end CM product may or may not be allergenic depending on how much PDS is retained. That said, given how widely the raw material from which PDS-01 is derived from and consumed worldwide, the inventors feel that the

issue of allergenicity may not pose a significant problem.

Table 3: Comparison of PDS with competing technologies.

Technology	PROS	CONS
Plant-Derived Serum Free Media (PDS)	• Very low-cost (\$\$1/litre) • Valorised origin • Readily available in SG	• Batch Variation (PDS-01 raw material is widely available in SG, but consistency of raw material may vary depending on processes by food companies from which PDS-01 is derived from – can be mitigated by building close relationships with a few key food companies) • Allergens (some people may be allergic to legumes) – end CM product may or may not be allergenic depending on how much PDS is retained
Specialized Defined Media with Growth Factor Supplementation	• Bath consistency • Optimization	• Costly to rely solely on a 100% defined media formulation for large CM scale-up (some stem cell media cost \$\$1/mL) • Recombinant growth factors will be costly to produce in bulk quantity • Media is typically customized for each cell line, so CM cells from different species will likely require a distinct assortment of growth enhancement factors
Insect-based (Umami Meats)	• Low-cost (estimated at \$\$2-3/litre)	• Insects need to be specially reared for consistency, harvested, and processed • Allergens (shellfish allergy) and Pathogens - end CM product may or may not be allergenic depending on how much serum replacement is retained
Detoxify and Replenish Spent Media	• Cost reductions due to economisation on fresh media • Optimization	• Technically very challenging, requiring costly automation, sensors, and engineering solutions • Requires additional infrastructure for media filtration and processing

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The **second** competing technology is a traditional reductionist approach which aims at discovering key growth factors and the nutritional needs of various CM cells. This strategy potentially provides the best opportunity for process optimization and consistency. However, it will be very expensive to rely on a fully defined media for large scale manufacture of CM products. Moreover, the cost of recombinant growth factor production at a large-scale would be very high as analytical grade reagents will still be needed to produce growth factors. The inventors feel that even with the economies of scale, the cost for these recombinant growth factors is likely to remain high. As culture media is also typically customized for each cell line, CM cells from different species will likely require a distinct assortment of growth enhancement factors, this will surely drive up the cost.

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The **third** competing technology is one which Umami Meats holds a proprietary patent for. It is a promising low-cost solution, but insects need to be specially reared for consistency, harvested, and then processed. There may be allergens in the sera as well (for example shellfish allergy) and pathogens from the insects. However, as in the case of PDS, the end CM product may or may not be allergenic depending on how much serum replacement is retained.

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The **fourth** approach is one which relies on detoxification and replenishment of spent media. This approach reduces media cost as there is economization on fresh media. However, it is technically challenging to detoxify and replenish spent media with nutrients and growth factors. This requires expensive automation, sensors, and customized engineering solutions. Additional infrastructure
5 for media filtration and processing to remove toxins and waste will be needed.

Hence, PDS of the present invention is the most viable and economical option for large scale cultivation of meat cells in cellular agriculture applications.

10 The above are exemplifying descriptions of the PDS replacement, in accordance with the present invention. It is foreseen that those skilled in the art can and will design alternative embodiments of this invention as set forth in the following claims.

CLAIMS

1. A plant-derived serum (PDS) replacement comprising one or more aqueous extract of a legume pulse or pulp thereof.
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2. The PDS replacement of claim 1, wherein the aqueous extract is obtained from Okara.
3. The PDS replacement of claim 1, wherein the legume is selected from a group consisting of soybean, chickpea, mung bean, lentil and quinoa.
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4. The PDS replacement of any one of claims 1 to 3, wherein the aqueous extract comprises plant albumin and/or plant transferrin.
5. A cell culture medium for culturing animal cells comprising the PDS replacement of any
15 one of claims 1 to 4.
6. The cell culture medium of claim 5, wherein the culture medium contains no animal-derived supplements.
- 20 7. Use of the PDS replacement of any one of claims 1 to 4 or the cell culture medium of claim 5 or 6 in cultivating animal cells.
8. Use of the PDS replacement of claim 2 or a cell culture medium comprising the PDS of claim 2 in cultivating fish cells.
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9. The use of claim 7 or 8, wherein the animal cells or fish cells are cultivated for meat.
10. A method of preparing a PDS replacement, comprising:
adding water to a legume pulse or pulp thereof to obtain a mixture;
30 blending the mixture; and
isolating a solution from the blended mixture to obtain a PDS replacement comprising

one or more aqueous extract of the legume pulse or pulp thereof.

11. The method of claim 10, wherein the step of adding water to a legume pulse or pulp thereof to obtain a mixture comprises adding water to Okara.

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12. The method of claim 10, wherein the legume is selected from a group consisting of soybean, chickpea, mung bean, lentil and quinoa.

13. The method of claim 10, wherein the water is added to the legume pulse or pulp thereof in a 4:1 mass ratio.

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14. The method of claim 10, wherein the solution is isolated from the blended mixture by filtering and/or centrifuging.

15. The method of claim 10, further comprising heating the legume pulse or pulp thereof, prior to mixing with water, to reduce antinutrient content.

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16. The method of claim 10, further comprising subjecting the solution to a bioprocess treatment.

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17. The method of claim 16, wherein the bioprocess treatment is an enzymatic digestion using a protease.

18. The method of claim 17, wherein the protease is subtilisin.

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19. The method of claim 16, wherein the bioprocess treatment is a fermentation process.

20. The method of claim 10, further comprising enriching an albumin and/or transferrin content of the solution.