

5

Prediction methods for fungal culture and characteristics prediction of mycelium derived products

10 Field of the invention

The present invention relates to a method for preparing fungal biomass comprising the step of cultivating at least one fungal strain according to conditions of fungal biomass cultivation, wherein said fungal biomass is characterized by a target property, wherein the method further comprises predicting how to
15 cultivate said fungal biomass to achieve said target property, and to relating prediction method. The present invention further relates to a method for preparing fungal biomass comprising the step of cultivating at least one fungal strain according to conditions of fungal biomass cultivation, wherein said fungal biomass is characterized by a target property, wherein the method further comprises predicting a target property of said fungal biomass based on the conditions of fungal biomass cultivation, and to
20 relating prediction method. The present invention further relates to biomass obtainable according to the methods of the present invention.

Background of the invention

25 There is a high demand in the food industry for edible fungi-based food products. Different fungal species are available worldwide, which possess different nutritional and organoleptic properties. They also require different growth conditions, which directs the object of this invention to understand and take advantages of all these parameters to develop new food products. However, manufacturing a fungal biomass and consequently a fungal species-based food product with a predicted composition, nutrition, taste and other
30 organoleptic properties is a challenging task. Therefore, this invention uses prediction methodology based on at least two previously trained mathematical models to help addressing these challenges, which will also enable to bring more versatility into consumable foods.

Relevant examples of prior art listed below focus on using one species or different strains of one species
35 and do not implement a multi-species, multi-side streams, multi-products approach as per this invention, wherein a multifunctional prediction pipelines are implemented, whereby single predictive models act as

building blocks of more complex prediction chains, hence providing answers to more than one question required by the product developer.

CN113502282 discloses a method for producing a pectinase by solid-state fermentation of penicillium, wherein the method involves high-throughput screening to predict a high-yielding strain *Penicillium* sp.Y for pectinase production and also optimizes the solid-state fermentation conditions to finally produce the pectinase preparation with an activity as high as 13800 U/g.

CN108624503 aims to provide a high-throughput screening method of a high-yield strain based on combination of a screening flat plate and a 96 shallow-well cell culture plate with -glucosidase, which can quickly and effectively improve the enzyme activity of -glucosidase produced by *Aspergillus niger*.

CN108739052 discloses a system and a method for optimizing edible fungus production parameters, aiming at the problems of difficult manual comparison difference, huge workload, low comparison efficiency, rough statistical result, difficult optimization experiment, inaccurate optimizing result and the like in the process of searching for the optimal culture environment and the optimal substrate based on the precondition of the maximum yield of edible fungi.

CN106651001 provides a method for predicting the yield of *Flammulina velutipes* by improving a neural network and an implementation system, which can predict the yield of *Flammulina velutipes* which are cultivated in a refrigeration house and have not completed the growth process.

Furthermore, Mark Shubert et al: *Appl Microbiol Biotechnol* 85, 703–712 (2010) uses a radial basis function neural network mode, which when trained with sufficient experimental data about the radial growth of the fungal strain, can predict its radial growth rate on petri dishes for pH, temperature, and water activity. This was validated for two fungal species.

Further similar prediction methods relevant for the methods of fungal culture are disclosed in CN 113 711 843 and Min Wang et al. (IUP: An intelligent utility prediction scheme for solid-state fermentation in 5G IoT. arXiv preprint arXiv:2103.15073, 28 March 2021).

Consequently, there is a need for a streamlined approach supporting on how to make or prepare an edible fungi species related food product with organoleptic properties and/or nutritional properties, or in more general terms, any fungal product with desired or targeted or target properties, via biomass fermentation,

specifically, via submerged fermentation or liquid-state fermentation. Particularly desirable are methods involving prediction pipelines that help plan biomass production experiments.

Summary of the invention

5

The present invention addresses the problem of reducing the use of natural resources by providing novel prediction means for use in the preparation of fungal biomass, and related method for preparing fungal biomass based on the predictions disclosed herein.

- 10 Accordingly, it was an objective technical problem of the present invention to provide methods for predicting at least one property of a fungal biomass based on provided conditions of the fungal cultivation, and to provide methods for predicting conditions of the fungal cultivation based on provided target or desired property of a fungal biomass. Particularly desirable are efficient prediction methods that allow for the broad prediction scope, at the same time reducing the need for extensive training data. The present
- 15 invention further provides methods for preparing the fungal biomass, based on the predictions according to the present invention.

The objective technical problem is solved by the embodiments disclosed herein and as characterized by the claims.

20

- The present inventors propose relying on the combination of multiple, at least two, previously trained mathematical models, wherein there is at least one regression model and at least one classification or clustering model, to generate predictions whose scope extend beyond that achievable with single predictive models. Accordingly, the models used may have been trained e.g. as follows: the regression
- 25 model has been trained using the output of the high-throughput screening (HTS) experiment(s) performed using fungal species, the clustering model or the classification model has been trained using data libraries comprising edible fungi information and side stream information. Said predictive mathematical models are logically linked using a compositional approach, whereby the prediction (output variables) of one model is used as the input variable of another model, thus enabling the effective utilization of different disjoint
- 30 training datasets (as exemplified above) to generate final predictions. It must be noticed that such an unprecedented approach allows decomposing complex prediction problems into less complex ones, and that through the cascading of different regression predictive models, combined with classification and/or clustering predictive models, the requirements on the amount of training data is greatly reduced. Moreover, composing/combining models through cascading allows developing flexible multifunctional

prediction pipelines, whereby the single predictive models become the building blocks of more complex prediction chains, which provide guidance for process optimization across the whole end-to-end food production process.

5 The invention will be summarized in the following embodiments.

In a first embodiment, method for preparing fungal biomass comprising the step of cultivating at least one fungal strain according to conditions of fungal biomass cultivation, wherein said fungal biomass is characterized by a target property, wherein the method further comprises predicting how to cultivate said
10 fungal biomass to achieve said target property, comprising the prediction steps of:

- a) providing a target property of the fungal biomass as an input;
 - b) predicting conditions of fungal biomass cultivation based on the input provided in a) using a computer implemented pipeline of at least two previously trained mathematical models, wherein the pipeline comprises the steps of
 - 15 b1) performing at least one intermediate prediction based on the input provided in a) using first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline, and
 - b2) predicting the conditions of fungal biomass cultivation by second of the at least two
20 previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1); and
 - c) outputting the predicted conditions of fungal biomass cultivation;
- wherein at least one of the at least two previously trained mathematical models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical models in
25 the pipeline is a clustering model or a classification model.

In a second embodiment, the present invention relates to a method for preparing fungal biomass comprising the step of cultivating at least one fungal strain according to conditions of fungal biomass cultivation, wherein said fungal biomass is characterized by a target property, wherein the method further
30 comprises predicting a target property of said fungal biomass based on the conditions of fungal biomass cultivation, comprising the prediction steps of:

- a) providing conditions of fungal biomass cultivation as an input;

- b) predicting a target property of the fungal biomass based on the input provided in a) using a computer implemented pipeline of at least two previously trained mathematical models, wherein the pipeline comprises the steps of
- 5 b1) performing at least one intermediate prediction based on the input provided in a) using first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline, and
- 10 b2) predicting the target property of the fungal biomass by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1); and
- c) outputting the predicted target property of the fungal biomass;
- wherein at least one of the at least two previously trained mathematical models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical models in the pipeline is a clustering model or a classification model.
- 15
- In a third embodiment, the present invention relates to a method for predicting conditions of fungal biomass cultivation, the method comprising the steps of:
- a) providing a target property of the fungal biomass as an input;
- b) predicting conditions of fungal biomass cultivation based on the input provided in a) using a computer implemented pipeline of at least two previously trained mathematical models, wherein the pipeline comprises the steps of
- 20 b1) performing at least one intermediate prediction based on the input provided in a) using first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline, and
- 25 b2) predicting the conditions of fungal biomass cultivation by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1); and
- c) outputting the predicted conditions of fungal biomass cultivation.
- 30 wherein at least one of the at least two previously trained mathematical models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical models in the pipeline is a clustering model or a classification model.

In a fourth embodiment, the present invention relates to a method for predicting a property of the fungal biomass, the method comprising the prediction steps of:

- a) providing conditions of fungal biomass cultivation as an input;
 - b) predicting a target property of the fungal biomass based on the input provided in a) using a computer implemented pipeline of at least two previously trained mathematical models, wherein the pipeline comprises the steps of
 - b1) performing at least one intermediate prediction based on the input provided in a) using first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline, and
 - b2) predicting the target property of the fungal biomass by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1); and
 - c) outputting the predicted target property of the fungal biomass;
- wherein at least one of the at least two previously trained mathematical models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical models in the pipeline is a clustering model or a classification model.

In a fifth embodiment, the present invention relates to a fungal biomass, obtainable according to the method of the present invention for preparing the fungal biomass.

In a sixth embodiment, the present invention relates to a food product, comprising the fungal biomass of the present invention or obtained using the fungal biomass of the present invention.

Brief description of figures

The invention is illustrated by the following drawings, which serve merely illustrative purposes and are not meant to be considered as limiting to the scope of the invention in any way, which is defined by the claims.

- Fig. 1** is an illustration of the overall setup and processes involved of this invention: the method (100) for predicting properties (e.g. conditions of fungal cultivation or properties of fungi-derived product, e.g. biomass) as disclosed in this invention, based on a target property inputted by the user (through User Interface – 107), to manufacture a fungal product, preferably a filamentous fungi-containing food product. Figure 1 embodies the exemplary setup of the invention, such as

the data available in public data sources (103), the data sourced from a high-throughput screening (HTS), optionally integrated with a robotic system connected with series of analytical instruments (104), and side-stream extraction processes (106). Screening in the HTS robotic system is preferably performed in multiple batches simultaneously in at least one culturing device, preferably in microtiter plate culture, solid state agar plate or small volume reactors (105). The analytical data generated through HTS robotic system (104), side-stream extraction (106), and the data collected from public sources (103) is forwarded to a processor configured to send the analytical data to the cloud computing server (102) and then to the cloud storage service (101). The desired outcome is produced by the application of multiple Machine Learning algorithms (108) (which embody previously trained mathematical model(s)), preferably of regression or classification or clustering type, trained with the information stored in the cloud storage service (101). The predictions generated by Machine Learning algorithms are processed by a processor and results are returned to the user for interpretation on the user interface device (107).

Fig. 2 illustrates clustering as described in Example 2.

Fig. 3 shows phylogenetic tree of a selected fungal Division, visualized as a sunburst diagram.

Fig. 4 presents biomass yield predictions (model predicted biomass concentration).

Fig. 5 shows diagram showing information flow for Example 5 of application.

Fig. 6 illustrates missingness matrix for MEAT food category entries (data from INRAN - Italian National Institute for Food and Nutrition).

Fig. 7 presents mapping between the nutritional profile of a fungal strain and food categories, provided by the NutrClassML model.

Fig. 8 shows diagram showing information flow for Example 6 of application.

Fig. 9 illustrates content of amino acids in side-stream extract versus severity of extraction process and reactor solid load.

Fig. 10 shows diagram showing information flow for Example 7 of application.

Fig. 11 demonstrates projection of texture dataset features (fermentation conditions) onto the 2-dimensional space of the first two PCA components, with marker shape associated to texture type.

5

Fig. 12 shows diagram showing information flow for Example 9 of application.

Fig. 13 shows exemplary implementation of the prediction method of the present invention. See Example 12 for details.

10

Detailed description of the invention

The invention will be described in detail in the following. It is to be understood that in absence of clear indication to the contrary, different features disclosed herein can be combined.

15

As mentioned before, in one embodiment the present invention relates to a method for preparing fungal biomass comprising the step of cultivating at least one fungal strain according to conditions of fungal biomass cultivation, wherein said fungal biomass is characterized by a target property, wherein the method further comprises predicting how to cultivate said fungal biomass to achieve said target property.

20

As referred to herein, the term “target property” of the fungal biomass preferably describes a property of said biomass which may be clearly defined (qualitatively or quantitatively) and which, in the context of the method of the present invention for preparing the fungal biomass, is a desirable property which is to be achieved when preparing the biomass according to the method. In other words, target property may also be called a desirable or desired property. The word “target” in the expression target property is to mean a property that is to be reached in the fungal cultivation efforts. As it is apparent from the description of the present invention, this target property may constitute input for predictions of the conditions of fungal biomass cultivation, which would lead the resulting biomass to be characterized by the target property. However, in alternative embodiments of the present invention, the conditions of fungal biomass cultivation can be used as an input, and a corresponding target property, as defined herein, that is obtainable in the biomass cultured according to particular conditions of fungal biomass cultivation, will be predicted. Further examples of target properties and their relevance will become apparent in the following.

25

30

Accordingly and preferably, the target property of the fungal biomass is selected from fungal strain,

nutritional profile, texture, yield, taste attributes, smell attributes, aroma attributes, mouthfeel attributes, consistency, edibility, and colour. Preferably, the target property of the fungal biomass is selected from fungal strain, nutritional profile, texture, yield and taste attributes. The invention further encompasses embodiments wherein the target property is more than one property selected from fungal strain, nutritional
5 profile, texture, yield, taste attributes, smell attributes, aroma attributes, mouthfeel attributes, consistency, edibility, and colour. Preferably, said target property is more than one property, selected from fungal strain, nutritional profile, texture, yield and taste attributes.

As understood herein, the fungal strain is preferably a property of fungal biomass is a fungal strain that
10 said fungal biomass comprises. Accordingly, said fungal strain is a property that includes information on the fungal strain, in particular includes biological classification of the fungal strain at issue. Exemplary fungal strains to be used in the method of the present invention will become apparent in the following description. In the methods of the present invention, if conditions of fungal biomass cultivation are provided as an input, a particularly suitable fungal strain that grows optimally in said conditions of fungal
15 biomass cultivation may be predicted. If the strain is used as an input, then, for example, optimal conditions of fungal biomass cultivation for said strain may be predicted. Whenever a reference is made to fungal strain, unless indicated to the contrary, it may be considered as reference to fungal species. Preferably, the fungal strain is filamentous fungus. As understood herein, when reference is made to a fungal strain by reciting a particular fungal species, any fungal strain belonging to said species would
20 preferably be understood to be encompassed. Further as referred to herein, the term strain, unless indicated to the contrary, also encompasses its plural version "strains", depending on the specific context.

As understood herein, the nutritional profile preferably comprises one or more properties selected from sugar content, amino acid composition, content of vitamin B12, content of metabolites, mineral content,
25 vitamin content, fiber content, fatty acid content, lipid content and protein content, preferably selected from sugar content, protein content and lipid content, or preferably selected from mineral content, vitamin content and fiber content. According to the present invention, a desired nutritional profile may be provided as an input for prediction. Alternatively, a nutritional profile that is obtained based on cultivation of the biomass in particular conditions of fungal biomass cultivation provided as an input may be predicted. The
30 properties that collectively may be considered to constitute the nutritional profile, as described herein, are defined in the following.

As understood herein, sugar content (also referred to as reducing sugar content or carbohydrate content) preferably refers to the %w/w of content of the biomass or the product of the invention, preferably

expressed with regard to the dry mass of said biomass or said product. The information on the sugar content may also include further details, e.g. content of complex and simple carbohydrates, including the breakdown with regard to pentoses or hexoses. The content of different types of sugars/carbohydrates, as referred to herein, may also be expressed in %w/w with regard to the total sugar/carbohydrate content
5 of the product or the biomass. Preferably, said content is determined according to DNS method.

As it is to be understood herein, in the methods of the present invention amino acid composition preferably refers to %w/w content of each amino acid with regard to the total amino acid content in the biomass or the product of the present invention. As it is known to the skilled person, in certain methods of amino acid
10 analysis it is not possible to distinguish between aspartate and asparagine, as well as between glutamate and glutamine, due to the hydrolysis conditions employed in the process. Accordingly, the content of Asp/Asn as well as the content of Glu/Gln shall be expressed as total content of the two amino acids in each of these pairs.

15 As it is to be understood herein, in the methods of the present invention the content of vitamin B₁₂, refers preferably to an amount of said vitamin, expressed in ng per g of the biomass, respectively.

As it is to be understood herein, in the methods of the present invention the content of metabolites refers to an amount of each metabolite in the biomass or in the product, expressed in ng per g of the biomass
20 or the product, respectively. It is to be noted that metabolite content should specify which metabolites are concerned. For example, metabolite content may refer to any of the metabolites selected from those known to the skilled person. Typically, metabolites refer to products of the metabolism of fungal species, as referred to herein. Exemplary metabolites include alcohols, amino acids, nucleotides, antioxidants, organic acids (e.g. acetic acid, lactic acid), polyols (e.g. glycerol) and vitamins. However, this list is not
25 meant to be construed as limiting.

As it is to be understood herein in the methods of the present invention the mineral content refers to the content of any of the minerals that are considered essential in human nutrition, which, for each of the minerals, may be expressed in ng per g of the biomass, referring to the dry mass of said biomass.
30 Preferably, minerals as referred to herein are selected from calcium, phosphorus, potassium, sodium, chloride, magnesium, iron, zinc, iodine, chromium, copper, fluoride, molybdenum, manganese, and selenium.

As it is to be understood herein, in the methods of the present invention the vitamin content preferably refers to content of a particular vitamin, referred to in ng/g of dry biomass. Vitamins are known to the skilled person and include vitamin A, vitamin B₁₂, vitamin B₆, vitamin C, vitamin D, vitamin E, vitamin K and vitamin O, among others.

5

As it is to be understood herein, in the methods of the present invention the fiber content preferably refers to %w/w content of dietary fiber in the dry biomass or the dry product.

As it is to be understood herein, in the methods of the present invention the protein content preferably refers to %w/w content of the protein in the dry biomass or the dry product.

10

As it is to be understood herein, in the methods of the present invention the fatty acid content preferably refers to %w/w content of fatty acid in the dry biomass or the dry product.

As it is to be understood herein, in the methods of the present invention the lipid content preferably refers to %w/w content of lipids in the dry biomass or the dry product.

15

In one embodiment, the nutritional profile preferably includes information on minerals, vitamins, protein, fat or lipids, carbohydrates, total fibers, insoluble fibers, soluble fibers, saturated fatty acids, monosaturated fatty acids, and polyunsaturated fatty acids. Accordingly, as understood herein the nutritional profile preferably includes information on at least one of minerals, vitamins, protein, fat or lipids, carbohydrates, total fibers, insoluble fibers, soluble fibers, saturated fatty acids, monosaturated fatty acids, and polyunsaturated fatty acids, more preferably it includes the information on minerals, vitamins, protein, fat or lipids, carbohydrates, total fibers, insoluble fibers, soluble fibers, saturated fatty acids, monosaturated fatty acids, and polyunsaturated fatty acids.

20

25

As it is to be understood herein, the texture can be measured using texture analyzer. Texture may also refer to texture diversity and/or texture attributes. Texture diversity preferably refers to texture types, relating to the type or the structure of the mycelium as identified by microscopy images and to various texture attributes. For example, a large pellet-type texture type (Type C) would result in a different shear force compared to a small pellet-type structure type (Type B). The various mycelial structural types are known to a skilled person to the art. Texture attributes are defined herein as in the density, cutting strength, shear strength/force, hardness, springiness (i.e., elasticity), cohesiveness, gumminess, chewiness, adhesiveness, firmness, spreadability, stickiness, puncture force, water release, water holding capacity

30

of the biomass (or the fungal-derived product, as applicable). As preferably understood herein, according to the present invention, the morphological and organoleptic properties are measured by a texture analyzer (for texture attributes), smell sensors (e.g. electronic nose), automated microscope, and/or image capturing device. These methods are conventional and known to the skilled person. Alternatively,
5 it is apparent to the skilled person that smell and taste may be evaluated through tasting panels.

As it is to be understood herein, in the methods of the present invention, the biomass yield (simply referred to as yield) preferably refers herein to the total yield of the biomass from a particular culture, expressed in g/L. It may also be normalized, e.g. according to the load of the reactor with the solid medium. The
10 expression "biomass concentration" may also be referred to the biomass yield expressed in g/L.

As preferably understood herein, taste attributes and smell attributes can be determined by tasting panels, composed of individuals that assess the taste and/or the smell of their provided samples. Preferably, tasting and smell panel are performed in parallel on several samples, and include certain reference
15 samples for normalization of the assessment. Accordingly, in an exemplary way of executing a tasting panel, each trained panelist is blindfolded and successively receives a sample. They define the sensory attributes they recognize in the samples, discuss the attributes together and choose common attributes that every panelist can associate to the same taste and aroma of the samples and reference samples to compare them. A second session is then started, and the panelists have to evaluate the samples
20 according to the chosen attributes and put a score, e.g. between 0 and 5, for each attribute. The session may be repeated on different days to increase statistical relevance of data and average of scoring may be calculated and plotted on a spider web. The taste attributes may be described as e.g. being sweet, sour, salty, bitter, umami, metallic, or astringent.

25 As preferably understood herein, the aroma attributes preferably related to aroma complexity, aroma intensity, aroma roundness and off-flavor.

As preferably understood herein, the mouthfeel attributes relate to juiciness and crumbliness.

30 A consistency, as preferably referred to herein in the context of the target property of fungal biomass relates to density and/or viscosity of said biomass. Consistency may also refer to elemental composition of the biomass.

Preferably, edibility is a Boolean variable indicating if the fungal biomass is edible or not.

The colour as referred to herein is preferably determined using the RGB system and a colour analyser at several positions, e.g., 20 different positions, on the samples. The mean values of these measurements are then used to compare the colour of the product. Preferably, a calibrated image capturing device is
5 used to determine the colour.

In the method of the present invention, predicting how to cultivate said fungal biomass to achieve said target property, comprises the prediction steps of:

- a) providing a target property of the fungal biomass as an input;
- 10 b) predicting conditions of fungal biomass cultivation based on the input provided in a) using a computer implemented pipeline of at least two previously trained mathematical models, wherein the pipeline comprises the steps of
 - b1) performing at least one intermediate prediction based on the input provided in a) using
15 first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline, and
 - b2) predicting the conditions of fungal biomass cultivation by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1); and
- 20 c) outputting the predicted conditions of fungal biomass cultivation.

The input of step a) as well as the output of step b) may be executed by any method known to the skilled person. For example, the input may be provided using the command line, or by providing a file with suitable instructions. The output may occur through creating an output file, or by displaying the values on
25 the screen of a computer. Accordingly, different ways of providing an input and receiving an output are known to the skilled person. Preferably, the input as well as the output, as understood herein, are both computer-implemented.

Step b) of the method of the present invention depends on the use of a computer implemented pipeline
30 of at least two previously trained mathematical models. Pipeline is to be understood as an arrangement, in the data and information flow of the method of the present invention, wherein one previously trained mathematical model generates, based on provided input, an output, which output is subsequently provided to a further previously trained mathematical model as an input. Said arrangement can also be described as a cascade or a prediction cascade.

The pipeline of the present invention comprises at least two previously trained mathematical models. In one embodiment, the computer implemented pipeline of at least two previously trained mathematical models includes exactly two previously trained mathematical models. However, in certain embodiments
5 of the present invention, the computer implemented pipeline of at least two previously trained mathematical models includes at least three, preferably at least four previously trained mathematical models.

It is to be understood that said previously trained mathematical model is preferably a machine learning
10 model. Any trainable mathematical model, preferably a regression or classification or clustering method, can be used in the methods of the present invention. The model describes the relationship between one or more predictor variables and a continuous response variable (regression), or maps a set of predictor variables into a property (classification), or identifies a similarity function between the modelled entities that can be used to group them into homogeneous groups (clustering).

15

More preferably, said previously trained mathematical model is a Linear regression, Bayes-Ridge regression, Support vector machines (SVM), K-nearest neighbours (KNN), Random forest (RF), Artificial Neural Network (ANN), K-Means, K-medoids, Fuzzy clustering model.

20 Linear model, as understood herein, is a supervised models (i.e. the learning is guided by examples of prediction) machine learning algorithms also known as linear regression model. Its learning phase is based on standard least square optimization for determining the best fitting linear function for the input data points. Even more preferably, said previously trained mathematical model is a Linear regression, K-nearest neighbors (KNN), Random forest (RF) K means or K-medoids.

25

Bayes-Ridge model, as understood herein, is a supervised regression technique that uses a regularization parameter for controlling the fitting errors introduced by multicollinearity among predictor variables.

Support vector machines (SVM), as understood herein, are supervised learning models that can be used
30 both for classification and regression. A support vector machine represents examples as points in space, and when trained determines a set of directions (the vectors) that separate the points into distinct categories divided by a clear gap that is as wide as possible. SVM natively support the identification of complex non-linear relationships between variables.

As understood herein, K-Nearest Neighbours (KNN) is a supervised machine learning approach that relies on the most basic assumption underlying all predictions: that observations with similar characteristics (predictors) will tend to have similar outcomes. KNN methods assign a predicted value to a new observation based on the plurality (for classification) or mean (sometimes weighted, for regression purposes) of its K "Nearest Neighbours" in the training set.

Random Forest (RF) is a supervised learning algorithm. The "forest" it builds, is an ensemble of decision trees, each tree representing a decision process that branches from a top root node to a decision leaf node, making at each branch a local choice based on the values of a single predictor variable. In the RF approach, many trees are built from data using a the "bagging" method (repeated sampling from the same dataset). The final prediction is generated from the opinions of decision trees, usually through a majority vote (for classification) or through averaging (for prediction).

As understood herein, an Artificial Neural Network (ANN) is a supervised learning algorithm that uses a feedforward network to generate a set of outputs from a set of inputs. An ANN is characterized by several layers of "perceptrons", units that mimic the functionality of neuronal cells. Each perceptron is trained with one data point at the time, and the input provided, causes an update of a mathematical function that encodes the relation between input and output variables. The output of each perceptron layer becomes the input for the next layer, until the final layer emits the output set of variables. ANN models can be used both for prediction and classification. For regression purposes, only one perceptron is in the final layer. For classification, one perceptron per class is in the final layer. One specific class of ANN is multilayer perceptron (MLP).

As understood herein, K-Means is an unsupervised machine learning approach (i.e. the learning is not guided by examples of prediction), which can be used for clustering a set of input multidimensional data points into K separate groups. According to the K-Means method, each cluster will be composed by the set of data points that have minimal distance from a "centroid" point, i.e. one that provides the reference for measuring similarity (usually, an Euclidian-like metric).

As understood herein, K-Medoids is another unsupervised machine learning that can be used for clustering a set of input multidimensional data points into K separate groups, therefore accomplishing the exact same task as K-Means. The most notable aspect of K-Medoids is the choice of centroid point. While K-Means uses centroids that are not in the dataset (they are in fact "average" points among those in the

cluster), each K-Medoid centroid is selected as one of the input data points, therefore providing for a better interpretability of the points in the cluster.

As understood herein, the Fuzzy clustering is the Fuzzy C-means clustering algorithm, an unsupervised
5 clustering algorithm for grouping data points into homogeneous classes. This approach has two major advantages over “hard” clustering methods, i.e. those methods that force one data point to belong to exactly one cluster (K-Means and K-Medoids): it automatically determines the number of clusters that best partition the data, and allows data points to belong to multiple clusters, a situation that naturally occurs in the domain of interest (e.g. a food can be both bitter and sweet).

10

In the method of the present invention, at least one of the at least two previously trained mathematical models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical models in the pipeline is a clustering model or a classification model. Accordingly, in one embodiment, at least one of the at least two previously trained mathematical models in the pipeline is a
15 regression model and wherein at least one of the at least two previously trained mathematical models in the pipeline is a clustering model. In one embodiment, at least one of the at least two previously trained mathematical models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical models in the pipeline is a classification model. Specific exemplary arrangements of the models in the pipeline used in the method of the present invention are apparent in
20 view of the following.

In step b) of the method of the present invention, the following steps are included: step b1) of performing at least one intermediate prediction based on the input provided in a) using first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an
25 input for second of the at least two previously trained mathematical models in the pipeline; and step b2) of predicting the conditions of fungal biomass cultivation by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1).

Accordingly, the output of the first used model, which is also an input of the second used model, may also
30 be referred to as intermediate prediction. Thus, in the method of the present invention, instead of directly predicting conditions of fungal biomass cultivation based on target property as an input, an intermediate prediction is performed, based on which the conditions of fungal biomass cultivation are predicted.

Preferably, in the method of the present invention, a previously trained mathematical model is applied. Accordingly and preferably, each of the models used in the present invention have already been trained. However, training of said mathematical model as part of the methods of the present invention is not excluded from the present invention. Accordingly, in one embodiment, the methods of the present invention may comprise the step of training the said mathematical model (i.e. each mathematical model used in the pipeline used in the methods of the present invention), so that the previously trained mathematical model is therewith obtained.

Preferably, in the method of the present invention, the regression model has been trained using the output of the high-throughput screening (HTS) experiment(s) performed using fungal species (see e.g. Figure 1). Preferably, as provided in the present invention, the fungal species (or the fungal strain) that is used to perform the HTS experiments is a filamentous fungus. Examples of filamentous fungus strains (species) are provided in the present application.

It is to be understood that said HTS involves a process. Said one process may for example refer to a biochemical process, a fermentation process, or any assay to be performed with the material obtained in the fermentation process (which is not to be considered to be particularly limited and may be a liquid fermentation process, for example submerged or surface liquid fermentation, or a solid-state fermentation process). Said process may involve the screening process of a fungus, or different fungi, growing on medium (or different media) obtainable in a process of extracting a lignocellulosic material – e.g. a side stream, i.e. an industrial or agricultural side stream.

Accordingly and preferably, the output of the HTS experiment(s) comprises one or more of fungal strain, sugar consumption, biomass yield, growth rate, protein content, cultivation conditions, cellulose content, hemicellulose content, carbon content, crude protein content, C:P ratio, moisture, mineral content, extraction conditions variables, texture type of biomass, shear force of biomass and nutritional profile of biomass.

As it is to be understood herein, in the methods of the present invention, sugar consumption preferably relates to change in time of the sugar concentration (preferably expressed in g/L) by the growing biomass as the growth progresses. As it is to be understood herein, such an analogous definition is applicable to any nutrient consumption, i.e. to any nutrient that can be consumed by the growing fungal biomass.

As it is to be understood herein, in the methods of the present invention, growth rate preferably provides information on growth of the biomass throughout the culture. It can e.g. be expressed as average value, maximal value, or provided as a function of time, e.g. in a graph. Preferably, the growth rate may be expressed in h^{-1} , and is preferably expressed through the doubling time, i.e. how long it takes for the
5 biomass in the culture to double its mass.

As it is to be understood herein, cultivation conditions are preferably further defined when discussing conditions of fungal biomass cultivation.

10 The following terms: cellulose content, hemicellulose content, carbon content, crude protein content, C:P ratio, moisture, mineral content, and extraction conditions variables, relate to the characterization of a particular screened medium obtainable in a process of aqueous extraction of a side stream (e.g. brewer's spent grain). Preferred processes of extracting side streams, in particular hydrothermal and liquid extraction of lignocellulosic materials – side streams, are disclosed in WO 2022/136708 and WO
15 2024/003323, which are incorporated herein by reference in their entireties.

A shear force or tensile strength, as referred to herein, preferably describes the ability of the biomass or the product (in particular food product) to resist unaligned forces applied to said biomass or said food product at their different parts and acting in different directions for the same weight. Preferably the forces
20 are applied only from the top. The applied forces are collinear, also known as compression forces. Preferably, it is expressed in N.

Preferably, as provided by the present invention the output of the HTS experiment(s) comprises one or more of sugar consumption, biomass yield, growth rate, protein content and cultivation conditions, and
25 shear force of biomass.

Alternatively, as provided by the present invention, the output of the HTS experiment(s) comprises one or more of cellulose content, hemicellulose content, carbon content, crude protein content, C:P ratio, moisture, mineral content, and extraction conditions variables. Accordingly, as defined herein the output
30 of the HTS experiment(s) relates to medium (or different media) obtainable in a process of extracting a lignocellulosic material and the process of extracting said lignocellulosic material.

It is preferred, in the methods of the present invention that the clustering model or the classification model, as referred to herein has been trained using data libraries comprising edible fungi information and side stream information.

- 5 As preferably it is to be referred to herein, the data libraries are clustered mined data libraries, wherein the data originates from the literature and public domain, and wherein similar data, or data characterized by similarity, are grouped together. In other words, data of similar characteristics is clustered, for example wherein the data as related genus or family or species having similar characteristics, or different species having similar characteristics, or a group of side streams or products sharing similar properties are
10 clustered together. Specifically, if species require a similar environmental growth conditions, said species are considered similar and therefore are clustered (or, in other words, grouped) together. Such step of clustering is in general recognizable to the skilled person.

- The term “mined” as defined herein means that the libraries are constructed using the data available to
15 the skilled person at the date of building the library, i.e. data scrapped from the literature and/or publicly available databases via literature data, published databases, and web scrappers, and further enhanced regularly through fed HTS data and laboratory experiments. Accordingly, the word “mined” refers to the process of collecting said data. Preferably, clustered mined data library may also be referred to as clustered data library, more preferably data library.

20

- Preferably, the edible fungi information is one or more selected from of fungal strain, edibility, fruiting season, lifestyle, habitat, seasonality, functional compounds, geographical location, availability of the fungal strain, substrate, climate, taste and/or smell, cultivation conditions, and nutritional profile of biomass. As provided in the present invention, the edible fungi information is not limited to mycelium of
25 filamentous fungi, but includes also the information on the fruiting bodies of said fungi. It is recognizable to the skilled person that certain prediction of the fungal biomass, which preferably is undifferentiated, i.e. free of spores or fruiting bodies, can be predicted based on data provided for e.g. said mushroom fruiting bodies.

- 30 Preferably, the fruiting season describes time span in terms of months of the year when a particular fungus is fruiting. As known to the skilled person, the fruiting season may depend on the geographical location. Accordingly and preferably, the term further includes the information on the geographical location.

Preferably, the lifestyle describes whether the fungus is symbiotic, saprotrophic or parasitic.

Preferably, the habitat describes where the particular fungus occurs in nature. This data may have the form of geographical location, or type of land, or it may be descriptive (e.g., found in forest, found in grassland or in anthropogenic landscapes, coast, desert, grassland, wetland, etc.).

5

Preferably, seasonality describes the months of the year at which the mushroom is fruiting.

Preferably, production of functional compounds includes information of compounds produced by the mycelium. Preferably, said functional compounds, which may also be referred to as active compounds, preferably refer to any substance with a beneficial (documented or proven) effect on a biological function, are preferably mycelium-derived active compounds selected from ergothioneine, lovastatin, ergosterol, resveratrol, glutathione, eritadenine, lentinan, and Concanavalin A. However, this list is not meant to be construed as particularly limited and further compounds produced in the mycelium, as recognized by the skilled person, may also be included. Exemplary compounds originating from *Pleurotus ostreatus* have been recently reviewed (Mishra et al., Int J Biol Macromol, 2021, 182, 1628-1637).

15

Preferably, the geographical location provides information on the geographical location of a particular fungal strain expressed in terms of continents or countries.

Preferably, the substrate as referred to herein provides comprehensive information about substrates, in particular preferred substrates wherein the fungus grows e.g., plant, tree, soil, etc.

20

Preferably, climate indicates climate preference of a particular fungus. This data is descriptive or numerical. Climate indicates the average climate condition (i.e. preferred temperature and atmospheric pressure ranges) around the time the fungi is fruiting, for example, average temperature during the fruiting season that is calculated during that time based on the geographical location of the edible fungi.

25

Preferably, taste and/or smell of the fungus are as defined hereinabove.

Preferably, the side stream information is one or more selected from extraction conditions for obtaining a medium, shelf-life, country of origin, industry of origin industry of use, yearly production volumes, lignin content, cellulose content, hemicellulose content, carbon content, nitrogen content, crude protein content, C:P ratio (carbon to phosphorus), C:N ratio (carbon to nitrogen), moisture, crude fiber content, fat content, ash content, nitrogen content, calorific value, density, information on typical usage, greenhouse gas

30

emissions of original product, mineral content, calcium content, phosphorus content, potassium content, sodium content, magnesium content, manganese content, zinc content, copper content, and iron content. More preferably, the side stream information is one or more selected from extraction conditions for obtaining a medium, shelf-life, lignin content, cellulose content, hemicellulose content, carbon content, nitrogen content, crude protein content, C:P ratio (carbon to phosphorus), C:N ratio (carbon to nitrogen), moisture, crude fiber content, fat content, ash content, nitrogen content, calorific value, density, mineral content, calcium content, phosphorus content, potassium content, sodium content, magnesium content, manganese content, zinc content, copper content, and iron content.

- 10 As it is to be understood herein, lignin content, cellulose content, hemicellulose content, carbon content, nitrogen content, crude protein content, crude fiber content, fat content, ash content, calcium content, phosphorus content, potassium content, sodium content, magnesium content, manganese content, zinc content, copper content, and iron content preferably refer to the content of lignin, cellulose, hemicellulose, carbon, crude protein, crude fiber, fat, ash, and minerals (calcium, phosphorus, potassium, sodium, magnesium, manganese, zinc, copper, and iron) in the side stream, preferably expressed as %w/w of the dry mass or in mg per g of the dry mass of the side stream.

- 20 As it is to be understood herein, C:P ratio preferably relates to the ratio of contents of carbon and phosphorus in the side stream, wherein said contents are as defined herein (preferably, as content of particular element in the material as expressed in g/kg of dry matter).

- 25 As it is to be understood herein, C:N ratio preferably relates to the ration of contents of carbon and nitrogen in the side stream, wherein said contents are as defined herein (preferably, as content of particular element in the material as expressed in g/kg of dry matter).

- As it is to be understood herein, moisture preferably relates to water content of the side stream, preferably given as %w/w.

- 30 Preferably, information on typical usage is descriptive data providing said information on side stream at issue. It may, among others, include the information on the industry of origin and/or industry wherein said side stream is used.

Preferably, greenhouse gas emissions of original product relate to CO₂ emission of the product or carbon dioxide equivalent, or CO₂ equivalent, abbreviated as CO₂-eq of the product, whose production led to the side stream at issue.

- 5 The side stream as understood herein may also preferably comprise a lignocellulosic material, in particular a lignocellulosic material originating from industrial and/or agricultural side stream. Lignocellulosic material is preferably herein defined as a material that comprises dry plant matter. Preferably, said lignocellulosic material comprises cellulose, hemicellulose and lignin. Preferably, the at least one lignocellulosic material is at least one industrial and/or agricultural side stream, as defined herein. Further
10 preferably, said lignocellulosic material is preferably solid or processed to be a powder before usage. As understood herein, the lignocellulosic material is preferably characterized by a particular colour, density, and/or mesh size distribution.

- The side stream material as encompassed by the present invention may be a lignocellulosic material
15 and/or an extract therefrom. Accordingly, the side stream information would preferably include its composition, the extraction conditions (or the pretreatment conditions, if applicable, in particular in the situations wherein no extraction of the side stream is foreseen), storage conditions, stability, place of origin and potential supplements added before or during or after cultivation on this side stream to increase its nutritional content if desired.

20

The side stream information may further include the information on the state of the side stream. Preferably, the side stream is a solid material. However, the side stream material may also be a liquid material.

- Examples of the lignocellulosic material, the information on which is preferably included in (ii), are spent
25 beer grain, spent grain, cereal brans, bagasse, cotton and oil press cakes from sunflower, hazelnut, shells and husks from nuts, grass and leaves waste, wood chips, coffee grounds, coffee husks, coffee silverskin, rapeseed and byproducts from the soy industry like soybean pulp ("okara"), banana leaves, banana peels, chicory roots, cassava peels, citrus pulp, cocoa, cocoa bean shell, cocoa mucilage, cocoa pod husks, coconut fibers, coconut husk, coconut shell, coffee pulp, corn cob, corn stover, cotton, cottonseed meal,
30 cotton seeds, hemp, spent hop, pea by-products, peanut hulls, peanut meal, peanut, potato peel raw, potato tuber, eucalyptus bark, Lantana weed, switch grass, rice bran, rice husk, rice straw, spent sugar beet, sugar beet pulp, sawdust, sugarcane bagasse wet, walnut shells, wheat bran, wheat distillers grains, wheat germ, wheat straw, lupin seeds, chickpea bran, chickpea pod husks, chickpea straw, olive waste, grape marc, pear pulp, sorghum bran, sorghum germ, sorghum stalk, sorghum straw, sunflower waste,

and/or tea waste. In addition, information on the peels or waste or pulp or pomaces of the following side streams are also preferably also included in data in (ii): oat, pine tree, dates, apple, apricot, spent barley, broccoli, cabbage, carrot, turnips, eggplant, kiwi, melon, alfalfa, pineapple, pomegranate, plum, watermelon, zucchini, asparagus, beetroot, cauliflower, garlic, onion, pumpkin, squash and/or tomato.

5 Examples of non-lignocellulosic materials, e.g. of proteinaceous materials the information on which is preferably included in (ii) are palm oil, sugarcane scum, molasses, whey, whey permeate, wool and silk. It is apparent to the skilled person that all the side streams as recited herein in addition to lignocellulosic material and/or proteinaceous material include sugars, minerals, and/or vitamins.

10 As provided by the present invention, the data libraries used for training of the classification models and/or clustering models may further comprise fungi-derived products properties and information. As preferably it is to be understood herein, fungal product (or the fungi-derived product) can be defined as fungal biomass or product containing fungal biomass. Preferably, and according to the invention, the fungi-derived products properties and information include information on taste, smell, edibility, colour, shear
15 force, tensile strength, consistency, mineral content, vitamin content, protein content, fat content, carbohydrate content, molecule and metabolite information, vitamin content, and compositional ingredients content. It is understood that this list also preferably include information on food product prototyping as in information on developed food prototypes as understood under meat analogues, dairy analogues, fish analogues, vegan and/or vegetarian foods, among others, preferably containing or based
20 on filamentous fungi or mycelium. Accordingly, inclusion of such information in the training set allows bridging the prediction of (target) properties of the fungal biomass with the properties of the fungal product. For example, nutritional profile as a target property of the fungal biomass can be correlated with mineral content, vitamin content, protein content, fat content, carbohydrate content, vitamin content, and/or fiber content of the fungal product (fungi-derived product) to be obtained using the biomass.

25 As referred to herein, food product based on filamentous fungi or mycelium preferably comprises at least 90 wt.% of fungal biomass (i.e., the fungal biomass obtainable according to the method of the present invention). As referred to herein, food product containing filamentous fungi or mycelium preferably comprises a mycelium in an amount of less than 90 wt.% (i.e., the fungal biomass obtainable according
30 to the method of the present invention). The wt.% in this particular case refers to wet %w/w content.

Preferably, in the methods of the present invention, compositional ingredients content is defined as content of substances/compounds that can be described as compositional ingredients. "Compositional ingredients" is preferably understood herein as supplemented preservatives, antioxidants and acidity

regulators, thickeners, stabilisers and emulsifiers, pH regulators and anti-caking agents, flavor enhancers, improving agents, stabilizers, thickening agents, colours, glazing agents and sweeteners, additives, aromatic compounds, and/or nutrients. It is understood that this list also includes nutritional profiles and physical properties of such ingredients (e.g. protein content, effects on shear force).

5

Preferably, as defined in the methods of the present invention, the conditions of fungal biomass cultivation comprise information on the culture medium and the fermentation process variables. More preferably, the conditions of fungal biomass cultivation consist of information on the culture medium and the fermentation process variables.

10

As referred to herein, the information on the culture medium preferably includes the concentration/amount of nutrients that must be present in the fermentation broth for growing the fungal biomass, such as preferred carbon source and sugars in culture medium, preferred nitrogen source, protein concentration, optimal C/N ratio, minerals, vitamins, amino acids, as well as information on the production of the medium, such as side stream information and extraction conditions. It is to be understood that depending on a particular embodiment of the invention, one, some or all of the properties described herein may constitute the information on the culture medium.

15

As referred to herein, the fermentation process variables include the settings of fermenter devices, such as temperature, pH, dissolved oxygen concentration, stirring or agitation speed, impeller type, and harvest time.

20

In one embodiment, the invention further relates to a method for preparing fungal biomass comprising the step of cultivating at least one fungal strain according to conditions of fungal biomass cultivation, wherein said fungal biomass is characterized by a target property, wherein the method further comprises predicting a target property of said fungal biomass based on the conditions of fungal biomass cultivation.

25

Accordingly, the predicting a target property of the fungal biomass, as referred to herein, comprises the prediction steps of:

30

- a) providing conditions of fungal biomass cultivation as an input;
- b) predicting a target property of the fungal biomass based on the input provided in a) using a computer implemented pipeline of at least two previously trained mathematical models, wherein the pipeline comprises the steps of

- b1) performing at least one intermediate prediction based on the input provided in a) using first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline, and
- 5 b2) predicting the target property of the fungal biomass by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1); and
- c) outputting the predicted target property of the fungal biomass.
- 10 It is to be understood that conditions of fungal biomass cultivation, target property, computer implemented pipeline of at least two previously trained mathematical models, as well as input and output, are as defined hereinabove.

In the method of the present invention, at least one of the at least two previously trained mathematical
15 models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical models in the pipeline is a clustering model or a classification model.

In step b) of the method of the present invention, the following steps are included: step b1) of performing at least one intermediate prediction based on the input provided in a) using first of the at least two
20 previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline; and step b2) of predicting the target property by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1).

25 Accordingly, the output of the first used model, which is also an input of the second used model, may also be referred to as intermediate prediction. Thus, in the method of the present invention, instead of directly predicting target property of the fungal biomass based on conditions of fungal biomass cultivation as an input, an intermediate prediction is performed, based on which the conditions of fungal biomass cultivation are predicted.

30

In the predictions methods of the present invention, the skilled person immediately recognizes that datasets used for training of the previously trained mathematical models (in particular selection of variables to be incorporated in the datasets, which preferably include – but is not necessarily limited to – at least some of target properties of the fungal biomass, as defined herein, and conditions of fungal

biomass cultivation, as defined herein) need to be so selected to allow for appropriate predictions as defined herein. Accordingly, the skilled person recognizes that there must be correlation between the variables/properties used in the prediction (as input, output, or intermediate prediction(s)) and variable in the dataset(s) used for the training of mathematical models. Further examples and explanations how the training datasets are to be selected and matched with the properties to be predicted are to be found in the Examples section, in particular in Examples 5 to 12.

In the method of the present invention for preparing the fungal biomass, step of cultivating at least one fungal strain according to conditions of fungal biomass cultivation is present. Fungal biomass cultivation is known to the skilled person. It is preferred in the method of the present invention that the cultivation is done as submerged fermentation.

As understood herein, submerged fermentation or submerged fungal fermentation is defined as cultivation of fungi in the liquid medium. As known to the skilled person, an alternative to submerged fungal fermentation is surface fungal fermentation, also referred to as solid state fungal fermentation. The liquid fungal fermentation medium that has been pH adjusted in solution or suspension is placed in an enclosed vessel, herein preferably a fermenter, which is usually sterilized to kill organisms that may interfere with fungal growth, according to the methods known to the skilled person. An inoculum of the at least one fungal strain as defined herein is introduced into the vessel (herein preferably fermenter) and, at least in the case of aerobic fungi, air is blown into the vessel. The contents of the vessel (fermenter herein) are preferably stirred according to the methods known to the skilled person, and preferably that can be integrated in the fermenter design. Stirring brings nutrients present in the medium and oxygen in continuous contact with the matter being fermented (herein the at least one fungal strain) and, preferably, temperature and pH are controlled at levels suitable to the fungus. After certain time, typically after between 1 to 12 days, depending on the type of fermentation, fungus, and exact fermentation conditions, among others, the fungal biomass can be harvested. (as noted by the skilled person, the timing as given herein may not necessarily apply to the cases of continuous fermentation). As however known to the skilled person, mixing may also be achieved by other methods than stirring, which may also influence the morphology of the fungal cells, as well as lead to subjecting the fungal cells to the shear stress. As understood herein, method of mixing is not meant to be limiting, and any applicable method known to the skilled person falls within the scope of the present invention.

The skilled person is capable of growing the fungal biomass according to the method of the present invention and according to the predictions of the present invention.

In the method for producing a fungal biomass by submerged fermentation of at least one fungal strain, the pH-adjusted fungal fermentation medium is provided to a fermenter suitable for growing fungal mycelium. Suitable fermenters are known to the skilled person. For example, a suitable stirred tank with
5 a specific stirrer useful in reducing the shear stress, or an airlift fermenter, is useful within the scope of the present invention. The fungal fermentation medium is apparent to the skilled person.

In one embodiment of the method of the present invention, the cultivation is done as solid state fermentation (or, in other words, surface fermentation).

10

The fungal strain to be cultivated (and to be subject to predictions, as described herein), is not meant to be particularly limited.

Preferably, the at least one fungal strain that is cultivated in the method of the present invention is selected
15 from Basidiomycota, Ascomycota, Pezizomycotina, Agaromycotina, Pezizomycetes, Agaricomycetes, Sordariomycetes, Pezizales, Boletales, Cantharellales, Agaricales, Polyporales, Russulales, Auriculariales, Hypocreales, Morchellaceae, Tuberaceae, Pleurotaceae, Agaricaceae, Marasmiaceae, Cantharellaceae, Hydnaceae, Boletaceae, Meripilaceae, Polyporaceae, Strophariaceae, Lyophyllaceae, Tricholomataceae, Omphalotaceae, Physalacriaceae, Schizophyllaceae, Sclerodermataceae,
20 Ganodermataceae, Sparassidaceae, Hericiaceae, Bondarzewiaceae, Cordycipitaceae, Auriculariaceae, and Fistulinaceae.

According to the present invention, the at least one fungal strain can be selected from the division Basidiomycota. Preferably, the at least one fungal strain selected from Basidiomycota can be a fungal
25 strain selected from the subdivision Agaromycotina. As defined herein, a fungal strain selected from the subdivision Agaromycotina can be a fungal strain selected from the class Agaricomycetes. Preferably, a fungal strain selected from Agaricomycetes can be a fungal strain selected from the order Agaricales, Auriculariales, Boletales, Cantharellales, Polyporales, and Russulales.

30 When the fungal strain is selected from the order Agaricales, the fungal strain is preferably selected from the family Agaricaceae, Fistulinaceae, Lyophyllaceae, Marasmiaceae, Omphalotaceae, Physalacriaceae, Pleurotaceae, Schizophyllaceae, Strophariaceae, and Tricholomataceae.

The fungal strain selected from Agaricaceae can be *Agaricus bisporus* or *Agaricus blazei*, more preferably

Agaricus bisporus.

The fungal strain selected from *Fistulinaceae* is preferably *Fistulina hepatica*.

- 5 The fungal strain selected from *Lyophyllaceae* is preferably *Calocybe indica*.

The fungal strain selected from *Marasmiaceae* is preferably *Lentinula edodes*.

The fungal strain selected from *Omphalotaceae* is preferably *Calvatia gigantea*.

10

The fungal strain selected from *Physalacriaceae* is preferably *Flammulina velutipes*.

More preferably, the at least one fungal strain selected from *Agaricales* can be a fungal strain selected from *Pleurotaceae*. Even more preferably, the at least one fungal strain of the present invention is a fungal strain selected from *Pleurotus pulmonarius*, *Pleurotus ostreatus*, *Pleurotus eryngii*, *Pleurotus citrinopileatus*, *Pleurotus florida*, *Pleurotus eunosmus*, *Pleurotus columbinus*, *Pleurotus ferulae*, *Pleurotus salmoneo-stramineus*, *Pleurotus sapidus*, and *Pleurotus salmoneostramineus*, still more preferably from *Pleurotus pulmonarius*, *Pleurotus ostreatus*, *Pleurotus citrinopileatus*, *Pleurotus florida*, *Pleurotus eunosmus*, *Pleurotus columbinus*, *Pleurotus ferulae*, *Pleurotus salmoneo-stramineus* and *Pleurotus salmoneostramineus*, even more preferably selected from *Pleurotus pulmonarius* or *Pleurotus ostreatus*, most preferably *Pleurotus pulmonarius*.

20

The fungal strain selected from *Schizophyllaceae* is preferably *Schizophyllum commune*.

- 25 The fungal strain selected from *Strophariaceae* is preferably a fungal strain selected from *Agrocybe aegerita* and *Hypholoma capnoides*.

The fungal strain selected from *Tricholomataceae* is preferably a fungal strain selected from *Hypsizygus tesselatus* and *Clitocybe nuda*.

30

Alternatively, a fungal strain selected from *Agaricomycetes* can be a fungal strain selected from the order *Auriculariales*, more preferably a fungal strain selected from the family *Auriculariaceae*. Preferably, a fungal strain selected from *Auriculariaceae* is *Auricularia auricula-judae*.

When the fungal strain is selected from the order Boletales, the fungal strain is preferably selected from the family Boletaceae and Sclerodermataceae. The fungal strain selected from Boletaceae is preferably *Boletus edulis*.

- 5 When the fungal strain is selected from the order Cantharellales, the fungal strain is preferably selected from the family Cantharellaceae and Hydnaceae. The fungal strain selected from Cantharellaceae can be *Cantharellus cibarius*. The fungal strain selected from Hydnaceae can be *Hydnum repandum*.

- When the fungal strain is selected from the order Polyporales, the fungal strain is preferably selected from the family Ganodermataceae, Meripilaceae, Polyporaceae, and Sparassidaceae.
- 10

- The fungal strain selected from Meripilaceae is preferably *Grifola* ²⁹*rondose*. The fungal strain selected from Polyporaceae can be from *Polyporus umbellatus* and *Laetiporus sulphureus* (L. *sulphureus*). The fungal strain selected from Sparassidaceae can be *Sparassis crispa*. The fungal strain selected from
- 15 Meruliaceae is preferably selected from *B. adusta* and *B. fumosa*.

- When the fungal strain is selected from the order Russulales, the fungal strain can be selected from the family Bondarzewiaceae and Hericiaceae. Preferably, a fungal strain selected from Russulales is a fungal strain selected from Hericiaceae, preferably selected from *Hericium erinaceus* and *Hericium coralloides*.
- 20 The fungal strain selected from Bondarzewiaceae can be *Bondarzewia berkeleyi*.

- According to the present invention, the at least one fungal strain can be selected from the division Ascomycota. Preferably, the at least one fungal strain selected from Ascomycota can be a fungal strain selected from the subdivision Pezizomycotina.
- 25

- The fungal strain selected from Pezizomycotina can be selected from the class Pezizomycetes. Preferably, the fungal strain selected from Pezizomycetes can be a fungal strain selected from the order Pezizales. Preferably, the fungal strain selected from Pezizales can be selected from the family Morchellaceae and Tuberaceae.

- 30 Preferably, the fungal strain selected from Morchellaceae is *Morchella esculenta*, *Morchella angusticeps*, *Morchella deliciosa*, *Morchella sceptrifomis*, *Morchella steppicola*, *Morchella punctipes*, *Morchella rufobrunnea*, *Morchella importuna*, *Morchella Jaurentinaa*, or *Morchella purpumsens*, preferably *Morchella esculenta*, *Morchella angusticeps* or *Morchella deliciosa*.

Preferably, the fungal strain selected from from Tuberaceae is *Tuber magnatum*, *T. estivum*, *T. uncinatum*, *T. indicum*, *T. rufum* or *T. melanosporum*, more preferably *T. melanosporum* and *T. magnatum*.

5

Alternatively, the at least one fungal strain selected from Ascomycota can be a fungal strain selected from the class Sordariomycetes.

Preferably, the fungal strain selected from Sordariomycetes can be a fungal strain selected from the
10 order Hypocreales.

The fungal strain selected from Hypocreales can be a fungal strain selected from the family Cordycipitaceae. The fungal strain selected from Cordycipitaceae can be a fungal strain selected from *Cordyceps militaris* and *Cordyceps sinensis*.

15

Alternatively, a fungal strain selected from Hypocreales can be a fungal strain selected from the family Nectriaceae. The fungal strain selected from Nectriaceae can be a *Fusarium* strain, for example *Fusarium venenatum*.

20 In another embodiment, the fungal strain selected from Sordariomycetes can be a fungal strain selected from the family Sordariaceae. The fungal strain selected from Sordariaceae can be a *Neurospora* strain, for example *Neurospora crassa*.

More preferably, the at least one fungal strain that is cultivated in the method of the present invention is
25 selected from *Agaricus* spp., *Aspergillus* spp., *Bjerkandera* spp., *Boletus* spp., *Calvatia* spp., *Cordyceps* spp., *Flammulina* spp., *Fusarium* spp., *Fomitopsis* spp., *Ganoderma* spp., *Grifola* spp., *Hericium* spp., *Hyphoroma* spp., *Inonotus* spp., *Laetiporus* spp., *Lentinula* spp., *Lepista* spp., *Morchella* spp., *Mycetinis* spp., *Neurospora* spp., *Paecilomyces* spp., *Panus* spp., *Pholiota* spp., *Pleurotus* spp., *Piptoporus* spp., *Polyporus* spp., *Rhizopus* spp., *Sparassis* spp., *Tremella* spp., *Trametes* spp., *Tricholoma* spp., *Tuber* spp., and *Wolfiporia* spp..
30

In one embodiment of the present invention, the at least one fungal strain that is cultivated in the method of the present invention is selected from mold species, preferably selected from *Aspergillus* spp., *Fusarium* spp., *Neurospora* spp., *Paecilomyces* spp., *Rhizopus* spp..

In an alternative embodiment, the at least one fungal strain that is cultivated in the method of the present invention is selected from non-mold species, specifically selected from Agaricus spp., Bjerkandera spp., Boletus spp., Calvatia spp., Cordyceps spp., Flammulina spp., Fomitopsis spp., Ganoderma spp., Grifola spp., Hericium spp., Hyphoroma spp., Inonotus spp., Laetiporus spp., Lentinula spp., Lepista spp., Morchella spp., Mycetinis spp., Panus spp., Pholiota spp., Pleurotus spp., Piptoporus spp., Polyporus spp., Sparassis spp., Tremella spp., Trametes spp., Tricholoma spp., Tuber spp., Wolfiporia spp..

More preferably, the at least one fungal strain that is cultivated in the method of the present invention is Agaricus spp., Bjerkandera spp., Boletus spp., Calvatia spp., Flammulina spp., Fomitopsis spp., Ganoderma spp., Hericium spp., Hyphoroma spp., Laetiporus spp., Lentinula spp., Morchella spp., Mycetinis spp., Pholiota spp., Pleurotus spp., Piptoporus spp., Polyporus spp., Sparassis spp., Tremella spp., Tuber spp., Wolfiporia spp..

Even more preferably, Agaricus spp., Bjerkandera spp., Boletus spp., Hericium spp., Laetiporus spp., Lentinula spp., Morchella spp., Pleurotus spp. or Polyporus spp., or Tremella spp..

In one embodiment, edible fungi co-cultivation or co-fermentation of such edible fungi with each other is used. In another embodiment, the previous embodiment is further combined with the usage of algae or bacteria or plant or archaea or animal cells/fat cells or a combination thereof. In one preferred embodiment, edible fungi (herein preferably understood as at least one fungal strain) is co-cultivated with algae selected from as Haematococcus pluvialis, Chlorella vulgaris and/or with cyanobacteria (e.g. spirulina). This allows more variability for the prediction models used in providing a desired solution for a desired output, for example, the predictive model suggests at least the usage of algae in combination with edible fungi, when setting a desired product with a fish-like taste rich in omega-3 fatty acids and/or vitamin B12.

The fungal strain to be cultivated, as well as the fungal strain to be used as input, output, or intermediate prediction in any of the methods of the present invention, is not meant to be particularly limited, as substantially any strain is encompassed by the present invention. As similar strains can be used as proxy of a particular strain in the prediction, the prediction and the cultivation may also extend to the strains for which the experimental data or literature evidence, necessary for training of previously trained mathematical models, is scarce.

The invention further relates to predicting how to cultivate a fungal biomass to achieve a target property, as taught and disclosed when considering the method for preparing the fungal biomass of the present invention, including any preferred embodiment and any preferred definition of said method. The invention further relates to predicting a property of the fungal biomass (which may also be referred to as target
5 property), based on the conditions of fungal biomass cultivation, as taught and disclosed when considering the method for preparing the fungal biomass of the present invention, including any preferred embodiment and any preferred definition of said method.

As understood herein, the present invention specifically recites and is directed to the method for
10 preparation of fungal biomass. However, it is apparent to the skilled person that the disclosed methods can be used or extended to different applications, such as the manufacturing of foods, foodstuffs, beverages, pharmaceutical, nutraceutical, as well as the methods of feed processing and other industrial applications. It is also apparent to the skilled person that the disclosed prediction methods can be extended to other properties related to the fungal biomass or the fungal biomass cultivation that are not
15 explicitly disclosed herein.

In one embodiment, the present invention relates to a fungal biomass, obtainable according to the method of the present invention for preparing the fungal biomass. The fungal biomass comprises at least one fungal strain. Said strain may be as defined hereinabove.

20

In one embodiment, the present invention relates to a food product, comprising the fungal biomass of the present invention or obtained using the fungal biomass of the present invention. The food product is not particularly limited. It may be for example a targeted food product or fungal derived products. Such food product may be manufactured as product derivatives from fungal cultivation, e.g. via using biomass and/or
25 the supernatant and/or any related extracts. Preferably, the food product of the present invention is a filamentous fungi-containing product. Said food product may be a solid food, or a beverage. However, encompassed are also products such as a pharmaceutical or a cosmetic product, and a nutraceutical health product.

30 The prediction methods of the present invention, and related methods for preparing fungal biomass, predicate on correlating target property of the fungal biomass and conditions of fungal biomass cultivation. However, the prediction methods may also rely on the fungi-derived product properties as an input or output, which can be correlated with the properties of the fungal biomass. Accordingly, the method of the present invention may further comprise a step of correlating the target property (properties) of the biomass

of the fungi-derived product properties that characterize the fungal product. Said fungi-derived product properties are preferably as defined hereinabove. selected from taste, smell, edibility, colour, shear force, tensile strength, consistency, mineral content, vitamin content, protein content, fat content, carbohydrate content, molecule and metabolite information, vitamin content, and compositional ingredients content.

5

Food products of the present invention preferably comprises meat, fish and dairy substitute products which are fully based on the fungal biomass, or comprising the fungal biomass of the present invention. It is noted that the foods or the food products prepared from the biomass of the present invention may be considered innovative foods (i.e. new or creative food categories), which are by no way limited to shapes, colours and compositions of natural meat products (or other known products) they were inspired by.

10

The meat-analogue or the meat-like food product is understood to preferably have a similar consistency or resemblance or texture or taste to the following animal meat in all its forms (breasts, fillets, thighs, ribs, wings, chunks, steaks, etc.), selected from: beef meat, poultry meat, fish meat, chicken meat, duck meat, goose meat, turkey meat, cow meat, pheasant meat, lamb and mutton meat, white meat, pork meat, ham meat, veal meat, deer or venison meat, seafood meat, prawn meat, crab meat, salmon, cod, pangasius, sardines, mussels and oysters.

15

Soft meat analogues are preferably understood as meat balls, sausages, fish fingers, tartar, minced meat, meat spreads, processed meat, Mett meat, luncheon meats, foie gras. Non-soft meat analogues are preferably understood as steak, beef jerky, burger patty, fillet, nugget, salami, whole-cuts, bacon, hot dogs, prosciutto, dried meat, and extruded products.

20

Soft dairy analogues are preferably understood as cream cheese, cheese spreads, processed cheese, whey cheese, pizza cheese, shredded mozzarella cheese, mozzarella cheese, soft cheese, semi-soft cheese, feta cheese, ricotta cheese, cottage cheese, camembert cheese, Roquefort cheese, Gorgonzola cheese, Brie cheese, blue cheese, Buchette cheese, goat cheese, quark, creams, coffee creamer, whipped cream, sour cream, milk chocolate spreads, margarine, butter, desserts, custard. Non-soft or hard dairy analogs are preferably understood as hard cheeses, semi-hard cheeses, cheddar cheese, parmesan cheese, etc.

25

30

The food product as understood herein may be a dairy product, for example cheese, yoghurt, drinkable yoghurt, milk drinks, milk, yoghurt, fresh cheese, whey cheese, cream cheese, medium-hard cheese, hard-cheese, and soft-mould cheese, and ice cream.

The food product as understood herein may also relate to different embodiments of seafood products, for example a crabcake, fishcake, tuna, salmon, or shrimp, as well as to different desserts, confectionery, or bakery goods, including chocolate, brownies, or cookies, flour, starch, bread, eggs, pasta.

5

The food product as understood herein may also relate to baked products including biscuits, cakes, pancakes, bread, rolls, muffins, donuts, brownies, cookies, pie crusts, pizza crusts, pie, tart, pre-made bread mixes, and sweet bakery mixes; snack foods including chips, pretzels, savory snack mixes, crackers, bubble tea boba, marshmallow, gelatins, sweet spreads, confectionary products, and sweetmeats; cereal products including cereal grains, cereal flakes, granola, muesli, and cereal bars; 10 alcoholic and non-alcoholic beverages, ready-to-mix beverages, beverage bases, sheeted baked goods, syrups, Kombucha, coffee, and tea; spice blends including seasoning preparations and spice mixes. Ready-to-heat foods or instant foods including soups, stews, noodle dishes, or frozen foods; desserts including ice cream, cakes, pies, tarts, custards, puddings, brownies, fondant, chewing gum, pastries, 15 mousse, frozen desserts, pie fillings, sweet fillings, toffee, and caramel; fat substitutes used in various food preparations and edible oils; colloidal foods including blancmange, custard, pudding, egg white foam, whipped cream, meringue, jam, jelly, margarine, mayonnaise, butter, and creamers; gluten-free bread chocolate bars, chocolate products, and chocolate-flavored snacks; dumplings; protein bars and shakes include protein-based bars and powdered shake mixes; extruded and extruded/puffed products like puffed 20 rice, crisped rice oats, and other extruded products.

In one embodiment, the present invention relates to a system configured for executing the prediction steps described herein. Such system is computer-implemented system, which are known to the skilled person.

25 The invention will be further illustrated by the means of the following exemplary embodiments.

In one embodiment of the invention the target property of the fungal biomass is a fungal strain. In this embodiment, the output of the step b1) is another fungal strain or a list of fungal strains characterized by similarity to the fungal strain of the fungal biomass. Based on the intermediate prediction of a strain (or a 30 list of strains), conditions of biomass cultivation are predicted that are suitable/optimal for said strain (or a list of strains, which may also be referred to as plurality of strains). It is to be understood that that similarity between fungal strains is intended to be a measure of the ability of fungal strains to grow on similar media. In this embodiment, the first mathematical model is a clustering model and the second previously trained mathematical model is a regression model. In this embodiment, in addition to the

conditions of biomass cultivation it is to be understood that the yield of fungal biomass cultivation is also outputted.

In one embodiment of the present invention, the target property of the fungal biomass is nutritional profile.

5 Accordingly, in this embodiment, the output of b1) is a fungal strain(s) (or a list of fungal strains, as applicable) with said nutritional profile. Thus, based on intermediate prediction of a strain with provided nutritional profile, the prediction of conditions for fungal biomass cultivation are made, which conditions are suitable for said strain. In this specific embodiment, the first previously trained mathematical model is a classification model, and wherein the second previously trained mathematical model is a regression
10 model. In step c), in addition to the predicted conditions of fungal biomass cultivation, the fungal strain of b1) may also be outputted, preferably is also outputted. It is to be understood that the yield of fungal biomass cultivation is also outputted.

In one embodiment, the target property of the fungal biomass is texture or taste attributes, and the output
15 of b1) is a fungal strain(s) with said texture or said taste attributes, respectively. Thus, based on said fungal strain(s) provided in b1), conditions for fungal culture are predicted that are suitable/optimal for growing said fungal strain. In this specific embodiment, the first previously trained mathematical model is a classification model, and wherein the second previously trained mathematical model is a regression model. In this specific embodiment, in step c) in addition to the predicted conditions of fungal biomass
20 cultivation, the fungal strain of b1) may also be outputted (preferably is outputted). It is to be understood that the yield of fungal biomass cultivation is also outputted.

In one embodiment, the target property of the fungal biomass is fungal strain, wherein the output of step b1) is optimal medium for said fungal strain, and wherein the conditions of fungal biomass cultivation
25 predicted in step b2) comprise (preferably consist of) information on the (at least one) side stream and extraction conditions for obtaining a medium suitable for the production of said fungal strain. Accordingly, as provided herein, the prediction using the regression model is followed by the prediction using a clustering model. In this specific embodiment of the present invention, the last model returns (i.e., outputs) at least one side stream in combination with its extraction conditions required to obtain the media
30 necessary for fungal growth (possibly a list of paired side streams with their respective extraction conditions).

In one embodiment, the conditions of fungal cultivation are predicted based on at least one of the properties: fungal strain, target nutritional profile, texture and taste based on at least two mathematical models, wherein there is at least one regression model and at least one classification or clustering model.

- 5 In one embodiment, the conditions of fungal cultivation and the yield associated therewith are predicted for a list of fungal strains using at least two mathematical models, wherein there is at least one regression model and at least one classification or clustering model.

10 It is to be understood that each of the predictions disclosed herein, either generically or as a specific embodiment, can also be reversed in a way that the output of the method becomes input and the input of the method becomes output. The present invention specifically relates to each prediction method disclosed herein, either generically or as a specific embodiment, as well as specially refers to each so reversed version of said methods.

- 15 For example, the present invention relates to an embodiment wherein the protein content of the biomass (i.e. of the mycelium) is predicted based on the conditions of fungal cultivation, provided as an input. Accordingly, in such a method, conditions of fungal biomass cultivation are provided as an input, and in step b1) the fungal strain which grows optimally at provided particular conditions is predicted. Then, in step b2) the nutritional profile, wherein the nutritional profile comprises (preferably consist of) protein content is predicted based on said fungal strain. However, based on the teaching provided herein, it is clear to the skilled person, that such a prediction can be reversed and based on the target property of the fungal biomass, herein nutritional profile comprising (preferably consisting of) protein content, conditions of fungal biomass cultivation are predicted. Therein, in step b1), a fungal strain matching the profile is predicted, and it follows that in step b2) conditions of fungal biomass cultivation suitable for the predicted strain are predicted. In step c), both the fungal strain and the conditions of the fungal biomass cultivation, as predicted in the method of the present invention, are outputted. It is to be understood that the yield of fungal biomass cultivation is also outputted.
- 20
- 25

In one embodiment, further prediction to correlate the properties of the biomass with the properties of the fungal product is added. For illustrative purposes only, without introducing limitations, the fungal product may e.g. be a meatball replacement product (a meatball) comprising fungal biomass. Herein, meatball nutritional profile may be provided as an initial input, which needs to be translated into nutritional profile of the fungal biomass as e.g. a target property. Based on this input, in the method of the present invention, in step b1) a fungal strain is predicted that matches the desired nutritional profile, to follow in step b2) with

30

the prediction of the conditions of fungal biomass cultivation. In one embodiment, if the nutritional profile as provided in the input cannot be achieved, a further prediction may be made to predict any compositional ingredients (defined hereinabove) in addition that need to be supplemented to the fungal biomass (already grown according to the method of the present invention) in order to reach the target nutritional profile in the final formulated food product.

The invention will be further illustrated by the following examples. These are not meant to limit the scope of the invention in any way, which is defined by the hereto appended claims.

10 **Examples**

Example 1: fungal strain information

As an example, the following data on *Pleurotus eringii* shown in the below table was retrieved through web scraping online sources and is part of the clustered data libraries (the information shown is only an excerpt show for the purposes of this example; in practical applications, more information and variables – for example nutritional profile or optimal cultivation conditions - is present for each fungal strain):

Species	<i>Pleurotus eringii</i>
Edibility	Edible
Fruiting season	Autumn
Continent	Europe, northern Africa, Asia (middle east)
Habitat	In association with plants (species Eryngium and others)
Lifestyle	Saprotrophic and carnivorous (nematodes)
Substrate	Lignocellulosic, supplemented with bran and gypsum
Climate	Temperate
Taste	Umami, meaty
Smell	Nutty, aniseed

20 **Example 2: clustered species**

Fungal species were clustered based on similar characteristics, specifically clustered when sharing a certain similarity related to the growth conditions. For example, cluster 13 groups together species living in grasslands in cold climates.

25

Example 3: side stream information

As an example, the following data on rice husks shown in the below table.

Product	Rice
Side stream	Husk
Lignin (%)	20-30
Cellulose (%)	35-40
Hemicellulose (%)	15-20
Carbon, C (%)	30-50
Crude protein, P (%)	2-6.7
C:P ratio	7.5-15
Moisture (%)	5-16
Crude fiber (%)	28-56
Fat (%)	2.5-3
Ash (%)	17-23
Nitrogen extract material (%)	28-30
Total phenolics (mg GAE/g)	14.2-15.6
Calorific value (MJ/Kg)	12-18
Bulk density (Kg/m³)	90-150
Typical usage	Waste, Bioenergy
Greenhouse gas emissions of original product (g CO₂/Kg product)	660-5690
Calcium (g/Kg dry matter)	0.3-1.7
Phosphorus (g/Kg dry matter)	0.3-4
Potassium (g/Kg dry matter)	1.7-7.3
Sodium (g/Kg dry matter)	0.1-0.4
Magnesium (g/Kg dry matter)	0.3-4.4
Manganese (mg/kg dry matter) – average	442
Zinc (mg/Kg dry matter) – average	43
Copper (mg/Kg dry matter) – average	2
Iron (mg/Kg dry matter) – average	56

Example 4: methodology

Pleurotus ostreatus was maintained at 24°C on malt peptone agar (MEA) comprised of malt extract 30 g/L, peptone 5 g/L, and microbiological grade agar 20 g/L. Mycelium cultures were run in deep-well
5 microtiter plates, in a culture broth containing phosphate buffer, metallic trace elements, an inorganic phosphorus and potassium source, sugar beet molasses as the main carbon source, and corn steep liquor as the main nitrogen source. Different carbon and nitrogen source concentrations are tested in each well. Each well is inoculated with a mycelium from an inoculum suspension of *Pleurotus ostreatus*. The plates are tightly closed with a lid allowing for air exchanges and incubated at 24°C with continuous shaking for
10 7 days. For each experimental run, the fresh inoculum is characterized; the number of colony forming units are measured on MEA plates. After cultivation, the biomass from each well is harvested, washed with demineralized water, dried, and weighed. Additionally, the biomass is processed after harvest for further analysis including biochemical assays. After washing, each biomass sample is lysed in a lysis solution containing Triton X-100. An example of an assay that can be performed on the whole cell lysate
15 include protein content determination using a commercial protein assay kit.

Example 5: predicting fungal biomass concentration and culture conditions based on inputted fungal strain

20 This example of application describes a use case whereby the invention can provide a prediction for the expected biomass yield (*property of biomass*) that would be produced in the culture of a certain fungal strain (*condition of the fungal culture*).

Given the following input parameters:

- 25 • a mushroom strain or fungal strain (denoted as **IN1**)

the invention will provide a reliable prediction of the following output parameters:

- an estimation of the average optimal biomass yield, expressed as concentration of dry biomass (denoted as **OUT1**)
 • the fermentation conditions (denoted as **OUT2**) that must be applied to obtain the biomass yield
30 **OUT1**.

The prediction will be based on trained Machine Learning models of two distinct classes:

1. A Clustering Machine Learning model (**ClustML**), which can predict the distance between two fungal species in terms of the nutrient composition of appropriate growth substrates. The model is trained on selected dimensions of the curated edible filamentous fungi clustered information libraries, specifically fruiting season, lifestyle, habitat, seasonality, geographical location, substrate, climate, phylogenetic information. The data of approximately 500 edible fungal species is currently included in the library, which covers both the Ascomycota and Basidiomycota divisions. Figure 3 shows a sunburst diagram view of the phylogenetic trees for a selected mushroom division (Ascomycota), for which data has been gathered. The collection of data for additional species and the enrichment of the clustered information libraries is constantly in progress. The **ClustML** model uses a Cluster-of-clusters approach to dominate the complexity of determining a suitable distance metric based on very diverse feature types. A t-Distributed Stochastic Neighbour Embedding algorithm has been used to generate the visualization of the fungal strain clusters for a subset of the species. The visualization is reported in Figure 2.
2. A Regression Machine Learning model (**RegrML**), which can predict the expected amount of biomass that can be produced through fermentation for a given fungal strain. The model is trained on the data obtained through the HTS platform for screening filamentous fungi and culture conditions, enriched with public information about tested growth conditions for edible mushrooms. This HTS knowledge base (i.e., HTS library) currently includes the quantitative outcomes of around 10,000 wet-lab experiments executed on the Mushlabs' HTS platform. The predictive model is based on a Linear regression, the main predictors including the concentration of nutrients in the culture media, the ratios among some key nutrients (notably concentrations of carbon/nitrogen sources), the oxygen transfer rate in the fermentation vessel, and temperature. An example of the biomass predictions that can be obtained for a fungal strain through the **RegrML** model is shown in the visualization given in Figure 4.

The diagram reported in Figure 5 describes the information flow that generates the output parameters from the input parameter. The fungal strain **IN1** is used to issue a query to the Clustering Machine Learning model **ClustML**, to retrieve a filamentous fungal strain (denoted as **SS**) among those present in the HTS Knowledge Base, which has the highest predicted similarity with **IN1**. The fungal strain **SS** is then used to query the Regression Machine Learning model **RegrML**, which will return the prediction for **OUT1**, the expected optimal biomass concentration for **SS**, as determined from the HTS data used to train the model, and the fermentation conditions (**OUT2**) to obtain the biomass concentration **OUT1**. As it

is to be understood herein, the biomass yield may also be expressed as concentration of obtained biomass.

As a side effect of the execution, data about the filamentous fungal strain identified by **IN1** will be added
5 to the HTS knowledge base, labelled with the “predicted” and “unconfirmed” tags. These labels may be later updated as new experimental evidence is collected.

Accordingly, parameters described as **OUT1** and **OUT2** can be used as input parameters, whereby the model will predict the **IN1** parameter as an output. Indeed, the same trained Machine Learning models
10 can be used within a more articulated algorithmic scheme to revert the prediction flow, i.e. to generate prediction about the set of fungal strains (output of the prediction model) whose mycelium could be grown by fermentation, for given fermentation conditions (input to the prediction model). Indeed, the **RegrML** model can be queried to obtain a prediction for the range of yields for all fungal strains, providing as an input the fermentation conditions. Such an output set can be filtered to produce a list of fungal strains that
15 could produce adequate yields, and this list used to retrieve from the **ClustML** model a larger set of filamentous fungi species whose mycelium could grow when the given fermentation conditions are applied.

Example 6: predicting suitable culture conditions for user-determined nutritional properties

20

This example of application describes a use case whereby the invention can provide a prediction for the fungal culture conditions (*condition of the fungal culture*) necessary to obtain a fungal biomass that matches a desired nutritional profile (*a property of a fungal biomass or a product*).

25 Given the following input parameters:

- a target nutritional profile of biomass (denoted as **IN1**), which will be expressed with reference to a specific food category (for instance “fish”) or sub-category (for instance, “cured meat”)

the invention will provide a reliable prediction of the following output parameters:

- a filamentous fungal strain (denoted as **OUT1**) whose mycelium can be produced via fermentation so to obtain a biomass that matches the target nutritional profile **IN1**
30
- an estimation of the average optimal biomass yield, expressed as concentration of dry biomass (denoted as **OUT2**) that can be obtained for strain **OUT1**
- a set of culture conditions (denoted as **OUT3**) to be applied for the fermentation of fungal strain **OUT1** so to achieve a biomass yield equal to **OUT2**.

The prediction will be based on trained Machine Learning models of two distinct classes:

1. A Nutrient Classification Machine Learning model (**NutrClassML**), which can map the nutritional profile of any specific food (or mushroom, or fungal biomass) with a set of food categories and sub-categories of interest. The model is trained on the data contained in the food and ingredients sections of the clustered information libraries, specifically on the features that provide the outcome measurements of food proximate analysis, i.e. total protein, fat contents (and % saturated), fibre (soluble and insoluble), carbohydrates (and % sugars), as well as details on amino acid content, fatty acid profile, minerals and vitamins of foods. Curated data for approximately 1,200 foods of interest is currently included in the library, and it includes the data about the nutritional profile of the fungal biomasses that have been screened for fermentation using the HTS platform. A large proportion of the data for foods and ingredients has been collected from authoritative sources (national Food&Nutrition authorities, and well reputed datasets such as FooDB). The visualization shown in Figure 6 shows an example of the raw (unprocessed) nutritional information (content of amino acids) for the foods in the meat category. The visualization is a tabular missingness map that describes the availability of amino acid content measurements for around 90 meat food products, as extracted from the Italian National Institute for Food and Nutrition public data repository. A KNN approach has been adopted for completing the missing entries of the dataset and reducing class imbalance, then a Random Forest model trained for providing the classification algorithm. From the voting of the trees, a similarity index of the input food profile against a set of selected food categories or subcategories can be obtained, as shown in Figure 7.
2. The Regression Machine Learning model (**RegrML**), already described in the previous example (Example 5 –predicting biomass yield).

The diagram reported in Figure 8 describes the information flow that generates the output parameter from the input parameter. The input nutritional profile **IN1** is provided as an input to the Nutritional Classification Machine Learning model **NutrClassML**, to determine a suitable fungal strain (denoted as **SS**), whose mycelium matches the desired profile, and whose fermentation has been characterized through the HTS platform. The logic of the processing continues as in the previous example, Example 5. The **RegrML** model will return for the fungal strain **SS** the expected optimal biomass yield and a set of possible fermentation conditions to achieve that yield.

Accordingly, the parameters described as OUT1, OUT2 and OUT3 can be used as input parameters to predict IN1. Indeed, the same trained Machine Learning models can be used within a more articulated
5 algorithmic scheme to revert the prediction flow, i.e. to generate prediction about which food products (output of the prediction model) could be produced, for given fermentation conditions, for instance nutrients contained in the media (input to the prediction model). Indeed, the **RegrML** model can be queried to obtain a prediction for the range of yields for all fungal strains, providing as an input the fermentation conditions. Such an output set can be filtered to produce a list of fungal strains that could produce the
10 highest yields, and this list used to retrieve from the **NutrClassML** model the list of food products that could be produced with the mycelia of the output filamentous fungal species, when the given fermentation conditions are applied.

Example 7: predicting side-stream based on inputted fungal strain

15

This example of application describes a use case whereby the invention can provide a prediction for the side-stream to be used for fungal culture (*condition of the fungal culture*) so to optimize the biomass produced through fermentation of a selected fungal strain (*property of a fungal biomass or a product*).

20 Given the following input parameters:

- a mushroom strain or fungal strain (denoted as **IN1**)

the invention will provide a reliable prediction of the following output parameters:

- a side-stream (denoted as **OUT1**) on which the mushroom strain **IN1** can be cultivated through fermentation
- 25 • a set of extraction conditions that should be used to process the side-stream **OUT1** and extract nutrients for the culture of fungal species **IN1**.

The prediction will be based on trained Machine Learning models of two distinct classes:

- 30 1. The Regression Machine Learning model (**RegrML**), already described in the previous example (Example 5 –predicting biomass yield).
2. A Clustering Machine Learning model for side-stream media (**ClustSSML**), which can map the nutrients in fermentation media with a set of side-streams whose extracts can be used for

filamentous fungi growth. The model is trained with data obtained from public sources (mostly scientific literature) and data acquired through the HTS robotic platform, where extracts from side-stream are analysed and tested for fermentation. The training data includes the characterization of amino acids, minerals and vitamins in the culture media, and the extraction conditions that can produce such extracts from side-streams. For example, Figure 9 shows the relation between the total amount of free amino acids in a side stream extract, while varying the extraction conditions. The **ClustSSML** model is based on a K-medoids clustering algorithm, and learns a similarity function between media, which is used to cluster side-streams and extraction conditions.

The diagram reported in Figure 10 describes the information flow that generates the output parameters from the input parameter. The fungal strain input **IN1** is used to query the **RegrML** model to obtain the characteristics of the optimal media (denoted as **OM**) for its growth. The characteristics of the desired media (preferably protein content, sugar content, vitamins, minerals and trace elements) are used to query the **ClustSSML** model, which will determine the side-stream (**SS**, i.e. **OUT1**) and the extraction conditions (**OUT2**) that can be used to generate a media that is similar to **OM**.

Accordingly, based on parameters **OUT1** as an input, **IN1** can be predicted. Indeed, the same trained Machine Learning models can be used within a more articulated algorithmic scheme to revert the prediction flow, i.e. to generate prediction about the set of fungal strains (output of the prediction model) whose mycelium could be grown by fermentation, for a given side-stream and extraction conditions (input to the prediction model). Indeed, the **ClustSSML** model can be queried to obtain a prediction of the nutrients in the extract that would be obtained for the given input side-stream and extraction conditions. The description of the medium predicted by the **ClustSSML** would then become the input to the **RegrML** model, which can return the set of edible filamentous fungal strains that can produce adequate biomass yields on that media (and also the fermentation conditions to obtain such yields).

Example 8: example on side-stream prediction

Starting with this example as well as in the following, it is noted that **A1** data represents data extracted from publicly available data (web scrappers, previous published literature data, etc.) related to fungal strain and side stream information, and **A2** data has been extracted from data generated via HTS screenings of filamentous fungi fermentation and characterization thereof (nutritional, organoleptic, etc.), side stream characterization and extraction processes, and food product prototyping and formulation analysis.

The following data on side-stream prediction shown in the below tables, provides an example of the predictions that can be obtained through the combined utilization of the **RegrML** and **ClustSSML** trained Machine Learning models.

5

The **RegrML** model receive as an input a fungal strain and produces as an output the information about the preferred media, detailing the nutrient composition. Additionally, the **ClustML** (the Machine Learning model that clusters edible fungal species according to the culture media they grow in, see clusters in Figure 2) can be also queried (using A1 data) with the IN1 fungal strain, to obtain an extended list of filamentous fungi species that grow on similar media.

10

Input variable (I1)	Value
Fungal strain (categorical)	Hohenbuehelia myxotricha
Output variables (O1 using A1 and A2)	
Medium composition:	
Preferred carbon source	Mannose/Glucose
Sugars in culture medium (g/L)	22-26
Preferred nitrogen source	Yeast extract/Soybean powder
Proteins in culture medium (g/L)	4-6
Minerals	-
Vitamins (mg/L)	Vit B6 (0.3)
Amino acids (mg/L)	-

The medium composition is used to retrieve a side-stream that can provide the required nutrient profile, from the similarity metric that has been learned by the **ClustSSML** model, as shown in the table below.

15 Minerals, vitamins and amino-acids are not used for the search, as they will be supplemented at fermentation time, as required.

Input variables (I1 = previous O1)	Value
Medium composition	
Sugar type	Mannose or Glucose
Sugar concentration (g/L)	22-26
Nitrogen source	Yeast extract or Soybean powder

Protein concentration (g/L)	4-6
Output variables (O1 using A1 and A2)	
Side-stream	Soybean cake
Output extraction conditions (O1 using A1 and A2)	
Extraction type	Hot-Water + Acid treatment
Temperature	165 °C
Retention time	55 minutes
Reactor solid load factor	9%
Acid treatment	Sulfuric acid
Acid concentration (w/w)	0.8

The **ClustSSML** model returns the best matching side-stream, in this example the soybean, and the conditions for extractions that result in the required amount of nutrients in the media.

5 A1 data:

- A1 data for fungal clustering: fungal strain, edibility, fruiting season, lifestyle, habitat, seasonality, geographical location, availability of the fungal strain, substrate and climate.
- A1 data for medium selection: fungal strain and cultivation conditions (to be specific in this case, preferred carbon source, preferred nitrogen source, optimal C/N ratio, amino acids in culture medium, minerals in culture medium, vitamins in culture medium, and fungal biomass yield from fermentation were used).
- A1 data for side stream selection: cellulose content, hemicellulose content, carbon content, crude protein content, C:P ratio, moisture, mineral content, and extraction conditions variables (to be specific in this case, pre-treatment process, extraction temperature, extraction pressure, extraction type, reactor load, retention time, and hemicellulose recovery rate were used).

A2 data:

- A2 data for medium selection: sugar consumption, biomass yield, growth rate, protein content and cultivation conditions (cultivation conditions same as A1 but coming from HTS experiments).
- A2 data for side stream selection: same as A1 but coming from HTS experiments in addition to carbon content (nitrogen content (in extract), and mineral content (in extract) (i.e. extracts composition).

Example 9: predicting fungal biomass texture based on inputted fermentation conditions

This example of application describes a use case whereby the invention can provide a prediction of the texture (*property of a fungal biomass or a product*) that will be obtained (for a given fungal species), when
5 a set of given fermentation conditions (*condition of the fungal culture*) are applied. We assume here that the fungal strain is known and fixed, i.e., it will not be subject to optimization.

Given the following input parameters:

- a set of fermentation conditions (denoted as **IN1**) to be applied for the production of mycelium
10 biomass for a fixed fungal strain;

the invention will provide a reliable prediction of the following output parameters:

- the expected texture type of the fungal biomass (denoted as **OUT1**), when the texture type is one
chosen from a predefined discrete set.

15 The prediction will be based on trained Machine Learning model of texture classification (**TextClassML**), which can map the fermentation conditions and fungal strains with a set a predefined classes that characterize relevant aspects of biomass texture. The relevant aspects of biomass texture, which are used for labelling the dataset used for training the model, include features that are evaluated through microscopy images resulting in various texture types (e.g. Texture A-C; Figure 11), and through texture
20 analysers evaluating the shear force expressed in Newton. The fermentation conditions comprise a comprehensive set of variables, which encompasses those required to fully describe the content of the media used for mycelium growth (concentrations of all nutrients in media, and amounts of trace elements), plus all physical parameters that are controlled during fermentation (temperature, dissolved oxygen, stirring speed, pH, among others). Also, they include the specific fungal strain, because the metabolic
25 responses of two different species of filamentous fungi may be quite different even for identical fermentation conditions. The total number of variables in the dataset used for training the model exceeds 50. A 2-dimensional visualization of the dependence between the predictors and the texture of the biomass is shown in Figure 11, which graphically reports the results of a Principal Component Analysis (first two principal components), for one specific fungal species. The texture of the fungal biomass has
30 been characterized into four distinct types, and the PCA clearly indicates the possibility of training machine-learning models that can efficiently separate classes based on the information contained in the input features. The classification model itself is built using a Random Forest classifier.

Another question that could be answered by combining the **TextClassML** classifier with other prediction models is the following one: what is the maximum biomass yield (denoted as **OUT1**), and the fermentation conditions (denoted as **OUT2**) to obtain it, for a given fungal strain (denoted as **IN1**) when a specific texture (denoted as **IN2**) is required? The diagram reported in Figure 12 describes the information flow
5 that would generate the output parameters from the input parameter.

The fungal strain **IN1** is used to query the **RegrML** prediction model, to retrieve a set of fermentation conditions (denoted as **CS**), which when used for culturing **IN1** would result in biomass yields that are all within the topmost quintile (or any other user-selected cutting threshold) of the predicted biomass yield
10 distribution. The set of conditions **CS** is used query the **TextClassML** model: for every condition (denoted as **C**) in the set, a prediction is generated for the texture of the biomass. The whole set of texture predictions is then filtered for selecting the fermentation conditions that result in the desired texture **IN2**, and among those in that subset, the fermentation condition that provides the highest biomass yield (**OUT2** and **OUT1**, respectively) are returned as output.

15 The diagram depicted in Figure 12 describes how the trained Machine Learning model **TextClassML**, which is built for providing a mapping between fermentation conditions (input features) and textures (predicted outcome), can be included in a more general algorithmic scheme that exploits the model to reverse the flow of the prediction, so that given as an input the desired texture, a prediction of suitable
20 fermentation conditions is obtained.

Example 10: biomass texture prediction

25 The following data on texture prediction shown in the below table, provides an example of the predictions that can be obtained through the **TextClassML** model.

Input variable (I1)	Value
Fungal strain (categorical)	Pleurotus eringii
Medium composition	
Preferred carbon source (categorical)	C5 sugars (xylose, arabinose, 70/30)
Sugars in culture medium (g/L)	25-28
Preferred nitrogen source (categorical)	Yeast extract
Proteins in culture medium (g/L)	4-6

Minerals	-
Vitamins	-
Amino acids (mg/L)	Arginine: 120
Fermentation control variables	
Dissolved oxygen concentration (% sat)	65%
pH	5.2
Agitation speed (rpm)	135
Impeller type (categorical)	Big mariner
Temperature (°C)	23
Output variables (O1 using A1 and A2)	
Texture type	Type B – small pellets
Shear force (Newton)	[14.2-15.6]

Given as input parameter a filamentous fungal strain, and as input variables those required to characterize the nutrients in the medium used for fermentation plus the variables that are controlled during fermentation and the choice of factors (such as impeller type) that are used in the fermenter equipment, the trained machine learning model will produce a prediction for the texture type, among a set of possible texture types. In the example, Type B texture is the predicted one, which corresponds to a biomass which is suitable to food applications where chewy, small particles are required, e.g. meatballs, sausages. The same input data is also conveyed to a regression prediction model (still a Random Forest), which will output an interval of predicted shear forces that would be obtained for the biomass through an objective texture analyser equipment.

A1 data

- fungal strain, cultivation conditions, and texture type of biomass.

A2 data

- fungal strain, cultivation conditions (preferred carbon source, sugars in culture medium, preferred nitrogen source, protein concentration in culture medium, amino acids in culture medium, minerals and vitamins in culture media, temperature, dissolved oxygen concentration, impeller type, agitation speed, pH, harvest time), texture type of biomass, and shear force of biomass.

Example 11: combined prediction and optimization for food application

The following tables shows an example of how the predictions generated by the trained Machine Learning models can be linked in optimization pipelines that support several steps of product development for mycelium-based food application.

- 5
- In this example, a novel food product that is characterized by a nutritional profile that matches the Pulses food category is considered. Mycelium will be the main ingredient (around 65%) of the food product, and the nutritional target must be satisfied by the fungal biomass. Further, the texture of the main ingredient fungal biomass must be of Type-C – large pellets, to provide the required mouthfeel.
- 10
- In a first prediction, the desired nutritional profile for the biomass is converted to a vector of nutrient amounts, that specifies ranges of amounts (per 100 grams) for amino acid, vitamins, minerals, and fatty acids that must be found in the fungal biomass. This step is supported by the data collected from food repositories (i.e. data A1), which allow to identify the “average pulse” nutritional profile, for this example the one provided in the table below:

15

English Name	Pulses – average profile
Energy (kcal)	343
Available carbohydrates (g)	53.6
Sugars (g)	2.6
Starch (g)	44.3
Protein - total (g)	25.2
Fiber - total (g)	7
Fiber - insoluble (g)	6.64
Fiber - soluble (g)	0.36
Lipids (g)	3
Saturated Fatty Acids (Fas) (%)	21.37
Monounsaturated FAs (%)	25.64
Polyunsaturated FAs (%)	53.85
C16:0 palmitic acid (%)	17.09
C18:0 stearic acid (%)	2.56
C18:1 oleic acid (%)	25.64
C18:2 linoleic acid (%)	49.57
C18:3 linolenic acid (%)	4.27

Calcium (mg)	90
Iron (mg)	5
Phosphorus (mg)	420
Niacin (mg)	2.6
Riboflavin (mg)	0.28
Thiamine (mg)	0.5
Vitamin A retinol equivalent (microg)	10

The nutritional profile is used to find the matching filamentous fungal strain from the **NutrClassML** mode. The model returns the output species *Agaricus brasiliensis* as the target species to produce the mycelial biomass, main ingredient for a food product with the desired nutritional profile. This species is then
5 provided as an input to the **RegrML** model, which returns the best fermentation medium and fermentation conditions, as detailed in the table below.

Input variables (I1)	Value
Fungal strain (categorical)	<i>Agaricus brasiliensis</i>
Output variables (O1 using A1 and A2)	
Medium composition	
Preferred carbon source	Glucose, or glucose/fructose
Sugars in culture medium (g/L)	18-21
Preferred nitrogen source	Yeast extract
Proteins in culture medium (g/L)	5.0-6.6
Minerals	-
Vitamins	-
Amino acids	-
Fermentation conditions	
Dissolved oxygen concentration (% sat)	75%
pH	5.5
Agitation speed (rpm)	160-180
Impeller type	Mariner
Temperature (°C)	25-28

The fermentation conditions are used to query the **TextClassML** model, to obtain a prediction for the texture of the biomass, as shown in the table below.

Input variable (I1)	Value
Fungal strain (categorical)	Agaricus brasiliensis
Medium composition (I1 = previous O1)	
Preferred carbon source (categorical)	Glucose, or glucose/fructose
Sugars in culture medium (g/L)	18-21
Preferred nitrogen source (categorical)	Yeast extract
Proteins in culture medium (g/L)	5.0-6.6
Minerals	-
Vitamins	-
Amino acids (mg/L)	-
Fermentation control variables	
Dissolved oxygen concentration (% sat)	75%
pH	5.5
Agitation speed (rpm)	160-180
Impeller type (categorical)	Mariner
Temperature (°C)	25-28
Output variables (O1)	
Texture type (categorical)	Type B – small pellets
Shear force (Newton)	[12.2-13.4]

- 5
- The predicted texture is not in line with the required one, and a search can then be performed for the fermentation conditions that can make the biomass to match to the desired morphology. The key factor is usually agitation, because the forces applied during fermentation have direct effects on the texture. The **TextClassML** model predicts that reducing the agitation speed from 160-180 to 130rpm will result in a Type-C texture. This change causes a slight reduction in the biomass, yield, as the sub-optimal stirring
- 10
- causes a predicted reduction of 5-7% in the biomass concentration (prediction from the **RegrML** model).

A1 data

- 15
- A1 data used for nutrient-mushroom mapping: food name, food category (includes a category named "mushroom" or "mycelium"), nutritional profile (in this case energy, carbohydrates, protein, fibers, fats, fatty acid profile, minerals, and vitamins were used).

- A1 data for fungal clustering (same A1 from example 8)
- A1 data for texture prediction (same A1 from example 10)

A2 data

- 5 • A2 data used for nutrient-mushroom mapping: fungal strain, nutritional profile (in this case energy, carbohydrates, protein, amino acid profile, total fibers, soluble fibers, fats, unsaturated fatty acids, fatty acid profile, minerals, vitamins, vitamin D, vitamin B12 were used)
- A2 data for medium selection (same A2 from example 8)
- A2 data for texture prediction (same A2 from example 10)

10

Example 12: exemplary implementation of the prediction method of the present invention

Figure 13 shows an exemplary implementation of the multi-model approach (pipeline of models or cascades of models) of the present invention, wherein the user selects a fungus (fungal strain) in the first panel (target property), then in the second panel the most preferred side streams to upcycle are outputted (regression model followed by clustering – see example 7), then the user selects one of the proposed side streams which triggers a prediction of the biomass cultivation conditions (regression model) as well as a prediction of the nutritional content of a potential food product comprising said fungal biomass (regression model for nutritional profile prediction followed by classification model for predicting possible matching food).

15

20

5

CLAIMS

- 10 1. A method for preparing fungal biomass comprising the step of cultivating at least one fungal strain according to conditions of fungal biomass cultivation, wherein said fungal biomass is characterized by a target property, wherein the method further comprises predicting how to cultivate said fungal biomass to achieve said target property, comprising the prediction steps of:
- 15 a) providing a target property of the fungal biomass as an input;
- b) predicting conditions of fungal biomass cultivation based on the input provided in a) using a computer implemented pipeline of at least two previously trained mathematical models, wherein the pipeline comprises the steps of:
- 20 b1) performing at least one intermediate prediction based on the input provided in a) using first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline, and
- b2) predicting the conditions of fungal biomass cultivation by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1); and
- 25 c) outputting the predicted conditions of fungal biomass cultivation;
- wherein at least one of the at least two previously trained mathematical models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical models in the pipeline is a clustering model or a classification model.
- 30 2. A method for preparing fungal biomass comprising the step of cultivating at least one fungal strain according to conditions of fungal biomass cultivation, wherein said fungal biomass is characterized by a target property, wherein the method further comprises predicting a target property of said fungal biomass based on the conditions of fungal biomass cultivation, comprising the prediction steps of:
- 35 a) providing conditions of fungal biomass cultivation as an input;
- b) predicting a target property of the fungal biomass based on the input provided in a) using a

computer implemented pipeline of at least two previously trained mathematical models, wherein the pipeline comprises the steps of

- 5 b1) performing at least one intermediate prediction based on the input provided in a) using first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline, and
- b2) predicting the target property of the fungal biomass by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1); and
- 10 c) outputting the predicted target property of the fungal biomass;
- wherein at least one of the at least two previously trained mathematical models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical models in the pipeline is a clustering model or a classification model.

- 15 3. A method for predicting conditions of fungal biomass cultivation, the method comprising the steps of:

- a) providing a target property of the fungal biomass as an input;
- b) predicting conditions of fungal biomass cultivation based on the input provided in a) using a computer implemented pipeline of at least two previously trained mathematical models, wherein the pipeline comprises the steps of
- 20 b1) performing at least one intermediate prediction based on the input provided in a) using first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline, and
- b2) predicting the conditions of fungal biomass cultivation by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1); and
- 25 c) outputting the predicted conditions of fungal biomass cultivation.
- wherein at least one of the at least two previously trained mathematical models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical models in the pipeline is a clustering model or a classification model.
- 30

4. A method for predicting a property of the fungal biomass, the method comprising the prediction steps of:

- a) providing conditions of fungal biomass cultivation as an input;
- b) predicting a target property of the fungal biomass based on the input provided in a) using a computer implemented pipeline of at least two previously trained mathematical models, wherein the pipeline comprises the steps of
- 5 b1) performing at least one intermediate prediction based on the input provided in a) using first of the at least two previously trained mathematical models, wherein output of said intermediate prediction constitutes an input for second of the at least two previously trained mathematical models in the pipeline, and
- 10 b2) predicting the target property of the fungal biomass by second of the at least two previously trained mathematical models in the pipeline based on the output of the intermediate prediction in b1); and
- c) outputting the predicted target property of the fungal biomass;
- wherein at least one of the at least two previously trained mathematical models in the pipeline is a regression model and wherein at least one of the at least two previously trained mathematical
- 15 models in the pipeline is a clustering model or a classification model.
5. The method of any one of preceding claims, wherein the conditions of fungal biomass cultivation comprise information on the culture medium and the fermentation process variables, preferably consist of information on the culture medium and the fermentation process variables.
- 20
6. The method of claim 5, wherein the information on the culture medium includes the concentration/amount of nutrients that must be present in the fermentation broth for growing the fungal biomass, such as preferred carbon source and sugars in culture medium, preferred nitrogen source, protein concentration, optimal C/N ratio, minerals, vitamins, amino acids, as well as
- 25 information on the production of the medium, such as side stream information and extraction conditions.
7. The method of claim 5 or 6, wherein the fermentation process variables include the settings of fermenter devices, such as temperature, pH, dissolved oxygen concentration, stirring or agitation
- 30 speed, impeller type, and harvest time.
8. The method of any one of preceding claims, wherein the target property of the fungal biomass is selected from fungal strain, nutritional profile, texture, yield, taste attributes, smell attributes, aroma attributes, mouthfeel attributes, consistency, edibility, and colour, preferably wherein the target

property of the fungal biomass is more than one property selected from fungal strain, nutritional profile, texture, yield, taste attributes, smell attributes, aroma attributes, mouthfeel attributes, consistency, edibility, and colour.

- 5 9. The method of claim 8, wherein the target property of the fungal biomass is selected from fungal strain, nutritional profile, texture, yield and taste attributes, preferably wherein the target property of the fungal biomass is more than one property selected from fungal strain, nutritional profile, texture, yield and taste attributes.
- 10 10. The method of claim 8 or 9, wherein the nutritional profile comprises one or more properties selected from sugar content, amino acid composition, content of vitamin B12, content of metabolites, mineral content, vitamin content, carbohydrate content, fiber content, fatty acid content, lipid content and protein content.
- 15 11. The method of any one of preceding claims, wherein the regression model has been trained using the output of the high-throughput screening (HTS) experiment(s) performed using fungal species.
- 20 12. The method of claim 11, wherein the output of the HTS experiment(s) comprises one or more of fungal strain, sugar consumption, biomass yield, growth rate, protein content and cultivation conditions, cellulose content, hemicellulose content, carbon content, crude protein content, C:P ratio, moisture, mineral content, extraction conditions variables, texture type of biomass, shear force of biomass and nutritional profile of biomass.
- 25 13. The method of claim 12, wherein the output of the HTS experiment(s) comprises one or more of sugar consumption, biomass yield, growth rate, protein content and cultivation conditions, and shear force of biomass.
- 30 14. The method of any one of preceding claims, wherein said clustering model or said classification model has been trained using data libraries comprising edible fungi information and side stream information.
15. The method of claim 14, wherein the edible fungi information is one or more selected from of fungal strain, edibility, fruiting season, lifestyle, habitat, seasonality, functional compounds, geographical location, availability of the fungal strain, substrate and climate, taste and/or smell, cultivation

conditions, and nutritional profile of biomass.

16. The method of claim 14 or 15, wherein the side stream information is one or more selected from extraction conditions for obtaining a medium, shelf-life, country of origin, industry of origin industry
5 of use, yearly production volumes, lignin content, cellulose content, hemicellulose content, carbon content, nitrogen content, crude protein content, C:P ratio (carbon to phosphorus), C:N ratio (carbon to nitrogen), moisture, crude fiber content, fat content, ash content, nitrogen content, calorific value, density, information on typical usage, greenhouse gas emissions of original product, mineral content, calcium content, phosphorus content, potassium content, sodium content,
10 magnesium content, manganese content, zinc content, copper content, and iron content.
17. The method of any one of claims 14 to 16, wherein the data libraries are clustered mined data libraries, wherein the data originates from the literature and public domain, and wherein the similar data are grouped together.
15
18. The method of any one of claims 1 to 17, wherein the target property of the fungal biomass is a fungal strain, and wherein the output of the step b1) is another fungal strain or a list of fungal strains characterized by similarity to the fungal strain of the fungal biomass.
- 20 19. The method of claim 18, wherein the first mathematical model is a clustering model and the second previously trained mathematical model is a regression model.
20. The method of any one of claims 1 to 17, wherein the target property of the fungal biomass is nutritional profile, and wherein the output of b1) is at least one fungal strain with said nutritional
25 profile.
21. The method of claim 20, wherein the first previously trained mathematical model is a classification model, and wherein the second previously trained mathematical model is a regression model.
- 30 22. The method of claim 20 or 21, wherein in step c) in addition to the conditions of fungal biomass cultivation predicted in b2), the at least one fungal strain of b1) is also outputted.
23. The method of any one of claims 1 to 17, wherein the target property of the fungal biomass is texture or taste attributes, and wherein the output of b1) is a fungal strain with said texture or said

taste attributes, respectively.

24. The method of claim 23, wherein the first previously trained mathematical model is a classification model, and wherein the second previously trained mathematical model is a regression model.
- 5
25. The method of claim 23 or 24, wherein in step c) in addition to the conditions of fungal biomass cultivation predicted in step b2), the at least one fungal strain of b1) is outputted.
- 10
26. The method of any one of claims 1 to 17, wherein the target property of the fungal biomass is fungal strain, wherein the output of step b1) is optimal medium for said fungal strain, and wherein the conditions of fungal biomass cultivation predicted in step b2) comprise information on the side stream and extraction conditions for obtaining a medium suitable for the production of said fungal strain, preferably consists of information on the side stream and extraction conditions for obtaining a medium suitable for the production of said fungal strain.
- 15
27. The method of claim 26, wherein the first previously trained mathematical model is a regression model and wherein the second previously trained mathematical model is a clustering model.
- 20
28. The method of any one of claims 1 to 27, wherein the computer implemented pipeline of at least two previously trained mathematical models includes exactly two previously trained mathematical models.
- 25
29. The method of any one of claims 1 to 27, wherein the computer implemented pipeline of at least two previously trained mathematical models includes at least three, preferably at least four previously trained mathematical models.
- 30
30. The method of any one of claims 1 to 29, wherein in c) yield associated with the conditions of fungal cultivation is also outputted.
31. The method of any one of claims 1 to 30, wherein the target property or conditions of fungal biomass cultivation outputted in step c) of the prediction constitute an input in step a) of the prediction in the method of any one of claims 1 to 30.
32. The method for preparing fungal biomass of any one of claims 1, 2 or 5 to 31, wherein the step of

cultivating at least one fungal strain according to conditions of fungal biomass cultivation is a submerged fermentation step.

33. The method for preparing fungal biomass of any one of claims 1, 2 or 5 to 32, wherein the at least
5 one fungal strain is selected from Basidiomycota, Ascomycota, Pezizomycotina, Agaromycotina, Pezizomycetes, Agaricomycetes, Sordariomycetes, Pezizales, Boletales, Cantharellales, Agaricales, Polyporales, Russulales, Auriculariales, Hypocreales, Morchellaceae, Tuberaceae, Pleurotaceae, Agaricaceae, Marasmiaceae, Cantharellaceae, Hydnaceae, Boletaceae, Meripilaceae, Polyporaceae, Strophariaceae, Lyophyllaceae, Tricholomataceae, Omphalotaceae,
10 Physalacriaceae, Schizophyllaceae, Sclerodermataceae, Ganodermataceae, Sparassidaceae, Hericiaceae, Bondarzewiaceae, Cordycipitaceae, Auriculariaceae, and Fistulinacea.
34. The method for preparing fungal biomass of any one of claims 1, 2 or 5 to 32, wherein the at least
15 one fungal strain is selected from mold species, preferably selected from *Aspergillus* spp., *Fusarium* spp., *Neurospora* spp., *Paecilomyces* spp., *Rhizopus* spp.
35. The method for preparing fungal biomass of any one of claims 1, 2 or 5 to 32, wherein the at least
20 one fungal strain is selected from non-mold species, specifically selected from *Agaricus* spp., *Bjerkandera* spp., *Boletus* spp., *Calvatia* spp., *Cordyceps* spp., *Flammulina* spp., *Fomitopsis* spp., *Ganoderma* spp., *Grifola* spp., *Hericium* spp., *Hyphoroma* spp., *Inonotus* spp., *Laetiporus* spp., *Lentinula* spp., *Lepista* spp., *Morchella* spp., *Mycetinis* spp., *Panus* spp., *Pholiota* spp., *Pleurotus* spp., *Piptoporus* spp., *Polyporus* spp., *Sparassis* spp., *Tremella* spp., *Trametes* spp., *Tricholoma* spp., *Tuber* spp., and *Wolfiporia* spp.
- 25 36. The method for preparing fungal biomass of any one of claims 1, 2, or 5 to 35, wherein at least one edible fungi is co-cultivated with algae, preferably selected from *Haematococcus pluvialis*, and *Chlorella vulgaris*, and/or with cyanobacteria.
37. A fungal biomass, obtainable according to the method of any one of claims 1, 2 and 5 to 36.
30
38. A food product, comprising the fungal biomass of claim 37 or obtained using the fungal biomass of claim 37.
39. The food product of claim 38, wherein the fungal biomass comprises at least one fungal strain

- selected from mold species, preferably selected from *Aspergillus* spp., *Fusarium* spp., *Neurospora* spp., *Paecilomyces* spp., *Rhizopus* spp, or wherein the at least one fungal strain is selected from non-mold species, specifically selected from *Agaricus* spp., *Bjerkandera* spp., *Boletus* spp., *Calvatia* spp., *Cordyceps* spp., *Flammulina* spp., *Fomitopsis* spp., *Ganoderma* spp., *Grifola* spp.,
- 5 *Hericium* spp., *Hyphoroma* spp., *Inonotus* spp., *Laetiporus* spp., *Lentinula* spp., *Lepista* spp., *Morchella* spp., *Mycetinis* spp., *Panus* spp., *Pholiota* spp., *Pleurotus* spp., *Piptoporus* spp., *Polyporus* spp., *Sparassis* spp., *Tremella* spp., *Trametes* spp., *Tricholoma* spp., *Tuber* spp., and *Wolfiporia* spp.
- 10 40. A system configured for executing the prediction steps described in any one of claims 1 to 31.