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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
02 November 2023 (02.11.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/208970 A1

(51) International Patent Classification:
C12P 1/02 (2006.01) *C12P 13/20* (2006.01)
A23L 27/00 (2016.01) *C12P 19/30* (2006.01)
A23L 31/15 (2016.01) *A23L 27/40* (2016.01)
A23L 33/185 (2016.01) *A23L 27/22* (2016.01)
C12N 1/14 (2006.01) *A23L 27/23* (2016.01)
C12P 13/14 (2006.01) *A23L 27/24* (2016.01)

(21) International Application Number:
PCT/EP2023/060864

(22) International Filing Date:
25 April 2023 (25.04.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
20225372 29 April 2022 (29.04.2022) FI

(71) Applicant: **NORDIC UMAMI COMPANY OY** [FI/FI]; Karamalmintie 2, 02630 Espoo (FI).

(72) Inventors: **KIVELÄ, Reetta**; Tapiolantie 24 as 1, 00610 Helsinki (FI). **MÄKINEN, Outi**; Joukolantie 3 A 1, 00610 Helsinki (FI).

(74) Agent: **BOCO IP OY AB**; Kansakoulukatu 3, 00100 Helsinki (FI).

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

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

Declarations under Rule 4.17:
— *as to the identity of the inventor (Rule 4.17(i))*
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

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

(54) Title: FERMENTED UMAMI-CONTAINING ENZYMATICALLY ACTIVE BIOMASS, UMAMI CONCENTRATE, UMAMI PASTE, SOLID UMAMI PRODUCT, SALT-FREE OR LOW-SALT UMAMI EXTRACT, LOW-SALT UMAMI PRODUCT, AND METHODS FOR PRODUCING THE SAME

(57) Abstract: To enable the use of more versatile the use of ingredients to make the substrate bed for solid-state fermentation, to produce umami- containing enzymatically active biomass: - A multi-component substrate system suitable for producing umami flavor compounds is generated by combining a substrate bed from a) a first amount of one or more wet ingredients, having a first moisture content, and b) a second amount one or more dry water-absorbing unboiled unsteamed ingredients, having a second moisture content that is smaller than the first moisture content, wherein the ratio between the first amount and the second amount is selected to result in the overall moisture content of the substrate bed to be between 39% and 63% by weight, preferably between 45% and 59% by weight. - The multi-component substrate system is inoculated with filamentous fungi, after which solid-state fermentation is carried out leading to hydrolysis and mycelial growth to convert the substrate bed to fermented umami-containing enzymatically active biomass. The application contains further independent method and product claims.

WO 2023/208970 A1

Fermented umami-containing enzymatically active biomass, umami concentrate, umami paste, solid umami product, salt-free or low-salt umami extract, low-salt umami product, and methods for producing the same

Field of the invention

The invention relates to umami-containing products and methods of producing the same.

Technical background

Food system is in crisis. As the environment is the driver and outcome of the food system, the crisis impacts food production in two ways: First, current food production accelerates climate change. Second, current food production practices are increasingly complicated by extreme weather conditions caused by climate change.

There is a scientific consensus that animal protein production is the most burdening part of food production. Also, recent research in health and nutrition shows that the single most significant change for improved public health is to increase the intake of plant foods. The shift from animal proteins to emerging new proteins from plant and microbial origin is urgent.

Various studies show that food liking is the most important driver of food consumption. Without pleasant taste, the urgently needed shift to emerging proteins is not possible. Future proteins, unlike the animal-originated proteins, generally lack umami, which is the fifth basic flavour and can be described as "meaty", "savoury" and "brothy". Umami also enhances the perceived saltiness of foods and enables salt reduction and reduces bitterness that is a challenge in plant-based foods (Wang et al., 2020).

Taste is a biosensor system that guides animals towards most beneficial foods. Sweetness indicates the presence or carbohydrates and thus energy that is available fast, bitterness indicates potential toxicity and umami indicates the presence of protein. Umami has also been shown to increase both appetite and satiety (Mouritsen 2016; Masic and Yeomans, 2014). Because of evolutionary adaptation, sweet and umami are the only flavours humans crave from birth. The lack of umami of the current new protein offering likely explains the reluctancy to reduce the consumption of animal based proteins, and this creates demand for sustainable sources of umami.

Umami flavour originates from free amino acids, nucleotides, and small peptides. Soy sauce type of fermented condiments, that are a major source of umami in Asian cooking, also contain a complex mixture of organic acids, alcohols, aldehydes, esters, furanones, pyrazines, and S-compounds (Diez-Simon et al. 2020). Animal based broths (incl. meat, chicken, fish, bone etc.), are the base and umami source of numerous dishes and food products around the world. Their flavor and depth originates from nucleotides, amino acids, salts and organic acids (Kranz et al., (2018)).

Food processors, food service professionals and home cooks all face the same problem: how to get umami and richness to plant based foods? Vegetable broths are widely used, but their flavor lacks depth, as their content of umami amino acids is far below taste recognition threshold (Mougin et al 2015). Available solutions are scarce: monosodium glutamate (MSG), yeast extract and soy derived products. MSG suffers from a very negative consumer perception and is generally avoided by food processors and food service professionals. The allergenicity of soy derived products limits their use in many production facilities. Also, compared to pure compounds, umami produced by fermentation is richer and more complex than its single components.

Soy sauce is a traditional umami containing condiment with a global market of around 40 billion USD in 2020. Now, when living the era of food system crisis, pristine soy as an ingredient for umami production is not reasonable. As known, soy is one of the four big crops in the world, and the areas of the fields are enormous, abled by the GMO species. This causes the loss of biodiversity, which may be considered as a threat for the environment. In addition, soya is usually cultivated in the most fragile ecosystems and soya is known as an allergen. Soy sauce also has very distinct flavour, aroma and colour. It is an important ingredient in Asian cuisine, but is utilisable in e.g., European and Continental cuisines.

Soy sauce is produced by fermenting soybeans or a mixture of soybeans and wheat with *Aspergillus* or *Rhizopus* fungi. The resulting biomass, *koji*, is mixed with salt solution and matured in the presence of halophilic yeasts and lactic acid bacteria. Products in the market still have traditional ingredients – soybean and wheat. Only few attempts to use other ingredients exists: JP2014110771A discloses the use of cracked and steamed peas instead of soybeans to produce allergen-free *koji*.

Chinese patent application publication CN109198585 A discloses that black sesame seed and bitter buckwheat soy sauce is manufactured from buckwheat powder, wheat bran and sesame seed press cake. Cereals are mixed into a dough and steamed before mixing with sesame seed press cake. Followed by liquid fermentation in high salt and additional enzymatic saccharification step.

Chinese patent application publication CN101744226 A discloses that production of fermented sauce from oat, bean and rice. Raw materials are soaked and boiled, then solid state fermentation by inoculation with a blend of bacteria, fungus and yeast. Liquid fermentation step at high temperature. The product is described as a brightly coloured, tasty, salty and sweet. Despite using ingredients other than soybean, in this technical background, pristine, non-circular raw materials are necessary. Pulses (pea, mung bean) are used dried and require energy and water intensive soaking and steaming processes in order to be usable.

A research paper reports the use of soy milk process residue, okara, in combination with soaked and steamed soybean (Lee et al, 2019)

Because of its production method, soy sauce has a very high salt content. Excessive salt intake is a tremendous health problem globally. In many countries, the daily salt intake is more than twice the recommended daily allowance and leads to cardiovascular disease including heart failure (He et al. 2011). Salt is used during or slurry fermentation stage to inhibit growth or harmful microbes that are a health risk or spoil flavour and aroma.

Sassi *et al.* (Sassi *et al.* 2021) refer to work of Santhirasegaram *et al.* who cooked pre-soaked soybeans either by using a pressure cooker with saturated steam at 0,8-1,0 kg/cm2 gauge pressure for 40-45 min or a continuous oven at 6-7 kg/cm2 gauge pressure for a short time (20-30 s). Further, they roasted wheat at 150 °C oven for 30-45 s and cracked it into small flour particles. Sassi *et al.* then refer further to work of Devanti & Gkatzionis who combined cooked soybeans and roasted wheat in trays with 0,05-0,3% w/w of fungal spores.

Japanese patent application publication JP2016086700A discloses a manufacturing method of soy sauce type of product with <4% salt by preparing koji from dry soybeans, wheat grains and Aspergillus oryzae, mashing it with water or saline solution, sterilising moromi mash and optionally inoculating it with yeast.

Objectives of the invention

It is a first objective of the invention to improve climate friendliness of umami production. This objective can be met with the method according to the independent method claim 1, particularly advantageously with the method according to dependent method claim 19, and with the parallel independent claim for a fermented umami-containing enzymatically active biomass according to claim 41.

A further objective of the invention is to increase the variety of umami containing products. This objective can be met with:

- I) The method of producing an umami concentrate, according to parallel independent claim 27, and the parallel independent product claim 42;
- II) The method of producing an umami paste, according to parallel independent claim 29, and the parallel independent product claim 43;
- III) The method for producing solid umami product, according to parallel independent claim 31, and the parallel independent product claim 44;
- IV) The method for producing salt-free or low-salt umami extract, according to parallel independent claim 32, and the parallel independent product claim 45;
- V) The method for producing a low-salt umami product, according to parallel independent claim 36, and the parallel independent product claim 46.

The dependent claims describe advantageous aspects of the methods and the products.

Advantages of the invention

According to the first aspect of the invention, the method of producing umami-containing enzymatically active biomass comprises the steps of:

- generating a multi-component substrate system suitable for producing umami flavor compounds [such as at least one of the following, or preferably all of the following: glutamic acid, aspartic acid, glutamyl, aspartyl containing peptides, nucleotides (inosine-5'-monophosphate, guanosine-5'-monophosphate)] by combining a -preferably soy and wheat free- substrate bed from a) a first amount of one or more wet ingredients, having a first moisture content which preferably is between 60% and 95% by weight, more preferably between 70% and 90% by weight, most preferably 80% and 85% by weight, and b) a second amount of one or more dry water-absorbing unboiled unsteamed ingredients, having a second moisture content that is smaller than the first moisture content and preferably between 1% and 30% by weight, more preferably between 5% and 20% by weight, most preferably 7% and 15% by weight, wherein the ratio between dry ingredients and wet ingredients is selected to result the moisture content of the substrate bed to be between 39% and 63% by weight, preferably between 45% and 59% by weight;

- inoculating the multi-component substrate system with filamentous fungi; and

- carrying out solid-state fermentation leading to hydrolysis and mycelial growth to convert the substrate bed to fermented umami-containing enzymatically active biomass.

With the expression "unboiled unsteamed", it is meant that no such cooking step is performed on the ingredient which substantially increases the moisture in the ingredient and/or that substantially reduces the water absorption capacity of the ingredient. Thus soaking, boiling, steam cooking are not performed on the water-absorbing unboiled unsteamed dry ingredient. Dry heat processing, however, such as baking or roasting, is still a possible treatment for the water-absorbing dry unboiled ingredients.

The advantage of the method is that in this manner, thanks to the substrate bed containing dry water-absorbing unboiled unsteamed ingredients, less energy may be necessary in the umami-containing enzymatically active biomass production method. In consequence, wet ingredients may be used without energy consuming drying process. The dry water-absorbing unboiled unsteamed ingredients are just mixed with the wet ingredients in a suitable ratio to each other, in order to adjust the moisture content of the mixture. After this, the mixture is heated, preferably by steaming, to reduce microbial load.

The efficiency of the method results mainly from the fact that since dry water-absorbing unboiled unsteamed ingredients are used without soaking, cooking or steam cooking processes they substantially maintain their water absorption capacity. Thus, they are necessary to bring down the moisture content of the wet bed material down to a suitable level. This improves the climate friendliness because energy consumption since it enables utilizing as wet ingredients even circular ingredients or other kinds of ingredients so far unsuitable for umami production. Further advantages are that in this manner, energy consumption during processing of the ingredients may be reduced, and furthermore the use of fresh water may be reduced.

Compared with the method disclosed in Chinese patent application publication CN109198585 A, with the present method it is not foreseen to make a steamed dough from dry materials but in the substrate bed dry water-absorbing unboiled unsteamed ingredients are used with which the moisture of the substrate bed is controlled such that wet ingredients having a high moisture content can be used in the substrate bed.

The preparation of the steamed dough complicates the process unnecessarily, causes additional investment needs and also decreases the climate friendliness of the process. Even more importantly, the dough preparation and steaming steps practically destroy the water-absorbing capability of the dry material, thus making it unsuitable to control the moisture content of the substrate bed. With the present method, as wet ingredients also ingredients having even higher moisture contents (the moisture content of the wet ingredients may be is preferably between 60% and 95% by weight, more preferably between 70% and 90% by weight, most preferably between 80% and 85% by weight) can be used by combining them with water-absorbing dry unboiled unsteamed ingredients.

Compared with Chinese patent application publication CN101744226 A, thanks to the substrate bed used in the present method, the water-absorbing dry unboiled unsteamed ingredients are neither soaked nor boiled.

The method in Sassi et al. uses pristine starting materials. Soybeans have after soaking moisture ca. 57% by weight and the moisture remains substantially the same after steaming or boiling (Yin-Zi Piao and Jong-Bang Eun, 2020). These starting materials are substantially easier to handle as wet materials, especially the wet materials that result from the food processing side streams (also "circular ingredients"). Such wet materials typically have moisture between 60% and 95% by weight, in some occasions between 70% and 90% by weight, and in selected applications between 80% and 85% by weight.

A consequence of all this is that the present method enables the use of circular ingredients as wet ingredients, the circular ingredients including challenging, wet side streams like fresh or frozen vegetable processing side streams, brewer's spent grain, plant milk or protein extraction by-products, broken legumes and beans, oil seed press cakes, bread and dough waste and rejected fruits and vegetables, vegetable and fruit processing by-products such as peels or rejected pieces, legume processing side streams with high (i.e. within the specified ranges typically 60-95% w/w, preferably 70-90% w/w, in certain situations 80-85% w/w) moisture content such as high moisture extrusion waste.

If the substrate bed is soy and wheat free, the method enables the production of umami products without using soybean or wheat that are major allergens.

The inventors have found out that with their method, umami-containing enzymatically active biomass may be produced even from food industry side-stream derived ingredients, instead or in addition to pristine ingredients, so contributing to the climate friendliness of umami production. Food industry side-stream derived ingredients and especially the so-called wet fractions, are at the moment considered waste because of their limited shelf life and difficult handling. The invention is based on the idea that the use of such wet fractions will become possible in the production of umami-containing enzymatically active biomass if the moisture content is brought down to a suitable range by combining the wet ingredients with dry ingredients with adequate water absorption properties.

The dry ingredients are preferably chosen to form a mixture of larger particles for aeration of the multi-component substrate system and smaller particles for absorbing water from the wet ingredients. In this manner, the growth environment for the fungal culture(s) may be optimized.

Preferably, the dry ingredients are selected based on their particle size. The water distribution within the multi-component substrate system may be controlled with the dry holding capacity of the dry ingredients. This is advantageously carried out by mixing, which may be performed with a sufficiently low power or speed to avoid grinding the ingredients that causes agglomeration of the substrate bed, i.e. the substrate bed becoming dough-like.

To ensure aeration conditions, between 0,5% and 40% by weight, preferably between 1% and 20% by weight, of the particles in the dry ingredients may have a size larger than 4 mm.

To ensure water holding capacity, the dry ingredients may be selected to contain 20 - 70%, preferably 30 - 60%, of particles having a diameter in the range of 0,75 to 3 mm.

Preferably, in the method, natural hydration properties i.e. water holding capacity of the ingredients of the substrate bed may be controlled. The controlling may be carried out by i) adjusting the particle size and ratio of dry/wet ingredients to control water holding capacity and/or ii) indirectly such that water holding capacity is increased by increasing share of non-starch polysaccharides containing ingredients.

The dry water-absorbing ingredients may be selected to have a water holding capacity of at least 100% of their weight. In addition, or alternatively to this, a part, preferably at least 20% of dry ingredients, may have water holding capacity of at least 150% of its weight.

The dry ingredients may be heated, preferably to above 85°C, to reduce microbial load, before combining with wet ingredients and inoculation with fungal spores or mycelia. In addition, or as an alternative, the wet ingredients may be heated in dry heat, preferably to above 75°C, to reduce microbial load, before combining with dry ingredients and inoculation with fungal spores or mycelia. Alternatively or in addition, the substrate bed may be heated (preferably by steaming) preferably to above 75°C or to above 85°C upon the wet ingredients have been combined (preferably by mixing) with the dry water-absorbing unboiled unsteamed ingredients, to reduce microbial load. The substrate bed is then allowed to cool before it is inoculated with fungal spores or mycelia.

The combined ingredients may be considered to be suitable for producing umami amino acids if:

i) the combined ingredients comprise at least 1400 mg/100 g glutamic acid,

ii) optionally, if in addition to i), the combined ingredients further comprise at least 18% by weight carbohydrates;

iii) optionally, if in addition to i) and preferably also to ii), the pH of the combined ingredients is in the range of 4,8 - 6,8.

The substrate bed may be fermented at 26 - 40 ℃ (or at 26 - 36 ℃) at relative humidity between 60 and 100% (or 80 and 100%) for 12 -

72 h (or for 15-24 h), which may be followed by 24-36 h preferably in the same conditions but under ventilation to prevent the substrate bed from overheating. Overheating of the substrate bed may be prevented by ventilation and/or movement.

The internal temperature of the substrate bed is, after the inoculation, preferably kept below 40℃, which may be ensured by turning the substrate bed periodically.

The solid-state fermentation may be tested for success a) by controlling the extent of proteolysis quantified as i) increase in primary amino nitrogen, ii) decrease of residual protein or iii) release of glutamic acid/glutamate and/or by b) testing for the release of reducing sugars from hydrolysis of starch and non-starch polysaccharides.

The particle size distributions may be controlled to have aerated conditions for at least one aerobic fungus used in the solid-state fermentation to increase enzyme activities for an improved flavor and flavor precursor production.

In the particle size distribution, the surface area to volume ratio may be controlled.

Preferably, the wet and the dry ingredients in the substrate bed are substantially unboiled and/or neither soaked nor steamed.

The dry and the wet ingredients may be derived from side streams from vegetable, legume, oilseed and/or cereal processing industries. This enables utilizing such side streams that are normally considered as waste. This may be considered more climate friendly than utilizing pristine ingredients as the dry and/or wet ingredients.

The dry ingredients and the wet ingredients may comprise at least one of the following: broken legumes and beans, oil seed press cakes, brewer’s spent grain, plant milk and protein extraction by-products, bread and dough waste, under-utilized milling fractions such as hulls, husk, seed coat, and bran and rejected fruits and vegetables, vegetable and fruit processing by-products such as peels or rejected pieces.

In addition or alternatively, the dry and/or wet ingredients may include at least one of the following:

i) non-soy legumes, such as pea, faba bean, lupin, lentil, chickpeas, brown beans;

ii) grains, such as oat, barley, rye, buckwheat, wheat quinoa, millet;

iii) combinations of different legumes from i) and/or grains from ii);

iv) dry legume processing side stream, preferably from milling, classification, extrusion processes;

v) fresh legume and/or vegetable processing side streams, such as classification waste from frozen pea or bean production, preferably including split or otherwise damaged fresh peas and their hulls;

vi) oilseed processing waste, such as hemp, rapeseed, sunflower, olive press cakes;

vii) other seed processing waste, such as press cakes from production of extracts; and

viii) food waste, such as bread and other cereal foods, processed vegetable or legumes containing foods.

The dry and/or wet ingredients may include side stream from one, two, three or more of the following:

- fresh pea processing, preferably damaged fresh green peas;
- dried peas, preferably whole, cracked or milled;
- dried faba beans, preferably whole, cracked or milled;
- cereal flours or flakes;
- milling side streams;
- bakery side streams;
- side stream from plant protein extrusion.

In the solid state fermentation, fungal genera used for include at least one non-toxic species of the following *Aspergillus, Rhizopus, Geotrichium, Penicillium, Lentinula, Pleurotus, Auricularia, Agaricus, Flammulina, Hericium, Clitocybe, Hypsizygus, Sparassis, Ustilago, Fusarium*. Preferably, at least *Aspergillus Oryzae* is used.

The fermented umami-containing enzymatically active biomass may be further processed into flavor products (FIG 1 as "production of flavor products"). The processing into flavor products is preferably carried out using a high ionic strength ripening step (HIS; described below in detail in the context of FIG 6) or a low ionic strength ripening step (LIS; described below in detail in the context of FIG 5).

The methods for processing fermented umami-containing enzymatically active biomass into flavor products may enable the production of neutral, nuanced, rich umami products for any type of cooking or food processing. The methods are preferably carried out on biomass that preferably is soy and wheat free. In this manner, they can be suited to produce umami-containing flavor products that are soy and wheat free.

In the method of producing an umami concentrate:

- fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to the first aspect of the invention, is mixed with 8 - 20% (preferably 10-17%) salt w/w aqueous solution (brine) and the resulting mash is ripened in liquid state in the presence of halophilic bacteria and yeasts, preferably for 1 to 24 months, preferably at ambient temperature such as 15-22 ℃;

- after ripening, the mash is pasteurized and insoluble dry matter is removed, such as by pressing, decanting, centrifuging or filtering, to produce an umami concentrate.

The umami concentrate may be packaged to be used as such or used in the formulation of seasoning products.

In the method for producing an umami paste:

- fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to any one the first aspect of the invention, is mixed with salt and unfermented legumes, cereal products and/or vegetables,

- optionally blended the mixture to produce a paste

- ripening in the presence of halophilic bacteria and yeasts, such as for 1 month to 12 months, preferably at ambient temperature, such as 15-22℃.

The ripened product may be pasteurized and formulated into seasoning products.

In the method for producing solid umami products: at least one of the following steps a. to d. is carried out.

a. umami-containing concentrate or extract produced by ripening fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to the first aspect of the invention, is dried into powder.

b. fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to the first aspect of the invention, is used to formulate a solid, gratable seasoning or food product that is optionally solidified, preferably using starches, fats and/or hydrocolloids.

c. fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to the first aspect of the invention, is dried into powder or chunks.

d. fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to the first aspect of the invention is used to formulate a solid, gratable seasoning and/or food product that optionally is solidified, preferably using starches, fats and/or hydrocolloids.

In the method for producing salt-free or low-salt umami extract:

- umami extract is produced by mixing fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to the first aspect of the invention, with water to produce a slurry;

- optionally adding endogenous enzymes;

- mashing the slurry so that it undergoes enzymatic processing; and

- pasteurizing and filtering the slurry.

The mashing may be carried out for 3 to 5 h (which is possible even without acidification) or for more than 5 h (which is possible with acidification), but in any case preferably less than 24 h, to avoid or suppress bacterial growth. Without acidification it is difficult to carry out the mashing for longer than 5 h because bacteria that may spoil the product start to increase rapidly.

The mash may be acidified, preferably using lactic acid bacteria.

The filtered slurry may be used as such as liquid seasoning product, dried, concentrated or mixed with other ingredients.

In the method for producing a low-salt umami product (which is preferably soy and wheat free): a salt free or low-salt umami extract preferably produced with the method presented above, is combined with an umami concentrate preferably produced with the method presented above.

The adjusting ratio between the salt free or low-salt umami extract and the umami concentrate, the salt content, color and flavor may be controlled.

Furthermore, flavor modulators -such as vegetables, fruits, herbs, mushrooms, algae- may be added before cooking to modify flavor profile.

The methods for producing salt-free umami products and the low-salt umami products described above enable the production of umami products with significantly lower salt contents than traditional soy sauce process. Compared with Japanese patent application publication

JP2016086700 A, the sterilizing step involved therein inactivates enzymes that are beneficial during ripening process.

The low salt umami product is preferably a liquid with which the flavor modulators is altered from neutral to resemble meat, chicken, fish or bone broth.

The resulting product may be used as base for stocks or as neutral flavoring for dishes and food products.

The fermented umami-containing enzymatically active biomass has been manufactured with the method presented above.

The umami concentrate has been manufactured with the method presented above.

The umami paste has been manufactured with the method presented above.

The solid umami product has been manufactured with the method presented above.

The salt-free or low-salt umami extract has been manufactured with the method presented above.

The low-salt umami product has been manufactured with the method presented above.

All these products are preferably soy and wheat free.

List of drawings

In the following, the method and the products are described in more detail with reference to the attached drawings, of which:

FIG 1 shows the process steps from solid-state fermentation to end products;

FIG 2 illustrates the fermentation process from solid-state fermentation to the end products;

FIG 3 shows the particle size distribution in the dry ingredients of five tested substrates;

FIG 4 are photographs of fermented biomass having (a) large particle size, (b) small particle size, and (c) intermediate particle size;

FIG 5 is a flow chart of the production of the salt free umami extract;

FIG 6 is a flow chart of the production of the salty umami extract;

FIG 7 is a flow chart of the production of the low salt umami products;

FIG 8 shows the increase of extractable glutamic acid during solid state fermentation;

FIG 9 shows the glutamic acid content of low ionic strength ripened umami extract processed using LIS method 1 (neutral, short process) and LIS method 2 (acidified, long process); and

FIG 10 shows the effect of particle size distribution on glutamic acid after solid state fermentation after 2 days, 10 days and 20 days.

Detailed description

There is a huge demand for clean label, non-soy and circular umami ingredients in both the food industry and consumer market.

Our invention is directed to improve circularity in the manufacturing of umami flavour products. Circularity may be improved by utilizing side streams from vegetable, legume, oilseed and cereal processing industries e.g. broken legumes and beans, oil seed press cakes, brewer’s spent grain, plant milk and protein extraction by-products, bread and dough waste, and rejected fruits and vegetables, vegetable and fruit processing by-products such as peels or rejected pieces.

Side streams vary in composition, texture, particle size and moisture, which brings challenges for a regular fermentation process, since the fungi used for solid state fermentation are very sensitive to growth conditions and cannot grow on such substrates.

Existing processing methods cannot be used for utilising a wide range of side streams. Our invention solves this problem by creating a multi-component substrate system that comprises one or more wet and one or more dry ingredients with specified particle size distributions and hydration properties. The existing methods utilise dry beans or grains, which are crushed and boiled in energy-intensive processes to open the substrates for the fermentative microbes (i.e. JPS5356393A Kikkoman, 1978) or soaked and steamed. Our method uses the existing side streams as such, utilising the natural hydration properties of ingredients. Hydration properties depend largely on composition, microstructure and particle size of plant materials. Especially high content of non-starch polysaccharides increases water holding capacity. Particle size also has an important role in creating aerated conditions for the aerobic fungi used in the solid state fermentation to create high enzyme activities for efficient flavour and flavour precursor production. There is a delicate balance between particle sizes, as larger particles have a lower surface area to volume ratio, which influences extraction of components from the material. Our invention utilises a mixture of larger particle for improved aeration of the substrate bed, and smaller particles that efficiently absorb water from wet ingredients.

The schematic diagram (FIG 1) shows the process of producing enzymes by filamentous fungi and using the enzymes for hydrolysis and further umami type flavour products. Ingredients are selected based on composition, particle size and water holding capacity. Dry component should contain 30-60% of particles in the range of 0,75 to 3 mm. No more than 20-40% of particles should be larger than 4 mm.

Water holding capacity of the mixture should exceed 100%. At least 20% of water absorbing dry unboiled unsteamed ingredients material should have water holding capacity >150%. Dry ingredients are heated above 85°C to reduce microbial load. Wet ingredients are heated above 75°C in dry heat to reduce microbial load.

FIG 8 shows the influence of the solid state fermentation step on amount of extractable glutamic acid in the substrate. These data shows that the fermented, enzymatically active biomass is enriched in umami.

FIG 3 shows the dry ingredients particle size distribution of five tested substrates. The three particle size distributions in the centre result in a good quality fermentation, while too large (Dry 4) and too small (Dry 1) particles of the substrates result in partial or no fermentation.

FIG 4(a) to (c) are photographs of fermented biomass. Large (a) and Small (b) particle sizes of the dry substrate result in inappropriate fermentation, while intermediate size (c) results in appropriate fermented biomass.

FIG 10 shows glutamic acid contents of HIS products produced with small, mixed and large particle after 2d (dark grey), 10d (light grey) and 20d (medium grey)

Referring to FIG 1 which discloses the preferred embodiment of the method of producing umami-containing enzymatically active biomass, which in FIG 1 has been labelled as "solid-state fermentation of side streams" after heating, dry and wet ingredients are combined and inoculated with fungal spores or mycelia. The final mixture has a moisture content in the range of 39-63%, at least 1400 mg/100 g glutamic acid, at least 18% of carbohydrates and pH in the range of 4,8-6,8. The mixture is fermented at 26-36℃ at RH=80-100% (RH stands for relative humidity) for 15-24 hours, followed by 24-36 h in the same conditions but under higher ventilation prevent the biomass bed from overheating. The internal heat of the biomass should not exceed 40℃ at any stage. This is prevented by proper conditions and turning the biomass bed periodically. The indication of a successful solid-state fermentation are extent of proteolysis quantified as an increase in primary amino nitrogen, decrease of residual protein or release of glutamic acid/glutamate. Another indicator is the release of reducing sugars from hydrolysis of starch and non-starch polysaccharides.

Possible ingredients include non-soy legumes (e.g. pea, faba bean, lupin, lentil, chickpeas, brown beans); grains (e.g. oat, barley, rye, buckwheat, wheat quinoa, millet) ; combinations of different legumes and/or grains; dry legume processing side stream (e.g. from milling, classification, extrusion processes); fresh legume and vegetable processing side streams (e.g. classification waste from frozen pea or bean production, incl. split or otherwise damaged fresh peas and their hulls); oilseed processing waste (e.g. hemp, rapeseed, sunflower, olive press cakes); other seed processing waste (e.g. press cakes from production of extracts); food waste (e.g. bread and other cereal foods, processed vegetable or legumes containing foods). Preferred raw materials are side stream from fresh pea processing (damaged fresh green peas), dried peas (whole, cracked or milled); dried faba beans (whole, cracked or milled), cereal flours or flakes, milling side streams, bakery side streams and side stream from plant protein extrusion.

Fungal genera used for solid state fermentation include but are not limited to non-toxic species *Aspergillus, Rhizopus, Geotrichium, Penicillium, Lentinula, Pleurotus, Auricularia, Agaricus, Flammulina, Hericium, Clitocybe, Hypsizygus, Sparassis, Ustilago, Fusarium*. Preferably, at least *Aspergillus* oryzae is used.

The fermented biomass can be processed into flavour products in various ways. These methods can be divided in two categories: high ionic strength ripening process and low ionic strength ripening process.

1. High ionic strength ripening processes:

1.1. Umami concentrate:

FIG 6 is a flow chart of the production of the salty umami extract i.e umami concentrate. A rich and aromatic umami concentrate is produced by mixing umami-containing fermented enzymatically active biomass with 10-18% salt solution and ripening in liquid state in the presence of halophilic bacteria and yeasts for 1 to 24 months at ambient temperature (15-25°C). After ripening, the mash is pasteurised, insoluble dry matter is removed by pressing, decanting, centrifuging or filtering. The umami concentrate can be packaged and used as such or used for formulation of different seasoning products.

1.2. Umami paste:

Ripening can be carried out in paste format by mixing with salt and unfermented legumes, cereal products or vegetables and ripening as paste (optionally) in the presence of halophilic bacteria and yeasts for 1 to 8 months at ambient temperature (15-22°C). After ripening, the paste can be pasteurised and formulated into paste-like seasoning products.

1.3. Solid umami products:

a. Umami concentrate can also be dried into powder.

b. Umami concentrate can be used to formulate a solid, gratable seasoning or food product. The product can be solidified using starches, fats or hydrocolloids.

c. Umami paste can be dried into powder or chunks.

d. Umami paste can be used to formulate a solid, gratable seasoning or food product. The product can be solidified using starches, fats or hydrocolloids.

2. Low ionic strength ripening processes:

2.1. Salt free umami extract

FIG 5 is a flow chart of the production of the salt free umami extract. Salt free umami extract is produced by mixing umami-containing fermented enzymatically active biomass with water and mashing at optimal temperatures of the desired enzyme activities (40-55°C). Optionally, endogenous enzymes can be added. The mash can optionally be acidified using lactic acid bacteria. Without acidification, mashing can be carried out for 3-5 h before spoilage (LIS Method 1). To enable more thorough hydrolysis and extraction, the mashing time can be increased up to 24 h by acidification (LIS Method 2). The resulting slurry is pasteurised and filtered. The resulting product can be used as such as liquid seasoning product, dried, concentrated or mixed with other ingredients.

FIG 9 shows the difference between salt free umami extract process without acidification (LIS Method 1) and with acidification (LIS Method 2) in terms of glutamic acid content

2.2. Low salt umami products

FIG 7 is a flow chart of the production of the low salt umami products. Low salt umami products can be produced by combining salt free umami extract (cf. point 2.1 above) and umami concentrate (cf. point 1.1. above). By adjusting ratio, the salt content, color and flavour can be altered. Flavour modulators (e.g. vegetables, fruits, herbs, mushrooms, algae) can be added before cooking to modify flavour profile. By using flavour modulators, the neutral umami liquid can be altered to resemble e.g. meat, chicken, fish or bone broths. The resulting product can be used as base for stocks or as neutral but rich flavouring for various dishes and food products.

Practical Examples

Example 1: Enzymatically active fermented substrate

Ingredients:

700 g fresh vegetable side stream

300 g gluten-free milling side stream

1 g fungal spores

Process:

Fresh vegetable side stream is heated to 75℃ to lower microbial count and cooled. Fungal spores are mixed with gluten-free milling side stream, followed by fresh vegetable side stream. The substrate mixture is mixed loosely to avoid formation of dough-like texture. The moisture content of the substrate mixture should remain below 59% at this stage. The substrate mixture is transferred on a large surface. The layer thickness should not exceed 5 cm. Fermentation is initiated by increasing the ambient temperature to 30℃ and keeping the relative humidity between 80 and 100%. The substrate bed is turned periodically to avoid overheating. After 20 h, ventilation is increased to allow evaporating water to be removed. The fermentation is carried out for a total of 48 h. The fermented substrate is gently mixed to break any lumps. The substrate is now ready to be used for applications.

Example 2: High ionic strength process

Ingredients:

450 g sodium chloride

2550 g water

650 g enzymatically active fermented substrate

Process:

Salt solution is prepared by mixing salt with water. The salt is allowed to dissolve under slow mixing. Enzymatically active fermented substrate is added and mixing is continued until the substrate is fully hydrated. Optionally, the mixture can be homogenized into an even slurry at this stage. The container is covered and stored at 19 ± 4℃. To avoid anaerobic processes, the slurry is mixed periodically. After desired flavour profile and colour are obtained, the slurry is pasteurized at 85℃ to inactivate enzymes and microorganisms. The pasteurized slurry is pressed using a belt press to obtain clarified liquid (15% solids) and press cake (37% solids). The liquid fraction is used for final product formulation as a source of intense umami.

Example 3: Low ionic strength process

Ingredients:

4500 l water

800 g enzymatically active fermented substrate

0,5 g lactic acid bacteria starter

Process:

Water is heated to 42℃ and enzymatically active fermented substrate is added under stirring. Stirring is continued to allow hydration of all particles. The mixture homogenized into an even slurry to maximize surface area. The temperature of the whole mixture is kept at 42℃ under periodical stirring. Lactic acid bacteria starter is then added. The slurry is kept at 42℃ until pH of the mixture has decreased to 5,5. Then, temperature is increased to 50℃. for 4 h. The total process time is generally 16 h but can be increased for more complete hydrolysis and flavor biotransformation. To end hydrolysis, the slurry is pasteurized at 85℃ and solid phase is removed from liquid phase using a belt press. The obtained clarified liquid can be used as such for product formulations.

Example 4: Dried umami product

Press cake from examples 2-3 is oven or drum dried at 60℃. The dried press cake is milled to various degrees to obtain salty, aromatic particles or powder. The resulting product can be used to substitute table salt. The product has lower salt content than regular salt and contains umami flavor. The product also contains fiber and protein, offering a healthier alternative to table salt.

Final words

It is obvious to the skilled person that, along with the technical progress, the basic idea of the invention can be implemented in many ways. The invention and its embodiments are thus not limited to the examples and samples described above but they may vary within the contents of patent claims and their legal equivalents.

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

References:

Diez-Simon, C., Eichelsheim, C., Mumm, R. and Hall, R.D., 2020. Chemical and sensory characteristics of soy sauce: A review. Journal of agricultural and food chemistry, 68(42), pp.11612-11630.

He, Feng J., Michel Burnier, and Graham A. MacGregor. "Nutrition in cardiovascular disease: salt in hypertension and heart failure." European heart journal 32.24 (2011): 3073-3080.

Kranz M, Viton F, Smarrito-Menozzi C, Hofmann T., 2018. Sensomics-based molecularization of the taste of pot-au-feu, a traditional meat/vegetable broth. Journal of agricultural and food chemistry, 10;66(1):194-202.

Masic U, Yeomans MR. 2014. Umami flavor enhances appetite but also increases satiety. The American journal of clinical nutrition. 1;100(2):532-8.

Mougin A, Mauroux O, Matthey-Doret W, Barcos EM, Beaud F, Bousbaine A, Viton F, Smarrito-Menozzi C., 2015. Impact of boiling conditions on the molecular and sensory profile of a vegetable broth. Journal of agricultural and food chemistry. 11;63(5):1393-400.

Mouritsen, O.G., 2016. Deliciousness of food and a proper balance in fatty acid compositionas means to improve human health and regulate food intake. Flavour, 5(1), pp.1-13.

Sassi *et al*, 2021, Recent progress and advances in soy sauce production technologies: A review J Food Process Preserv. 2021;45:e15799. https://doi.org/10.1111/jfpp.15799

Wang, Wenli, Xirui Zhou, and Yuan Liu. "Characterization and evaluation of umami taste: A review." Trac Trends in Analytical Chemistry 127 (2020): 115876.

Yin-Zi Piao and Jong-Bang Eun. Physicochemical characteristics and isoflavones content during manufacture of short-time fermented soybean product (cheonggukjang), in J Food Sci Technol. 2020 Jun; 57(6): 2190-2197. Published online 2020 Mar 12. doi: 10.1007/s13197-020-04255-2

Claims:

1. Method of producing umami-containing enzymatically active biomass, **the method comprising the steps of:**

- generating a multi-component substrate system suitable for producing umami flavor compounds -such as at least one of the following, or all of the following: glutamic acid, aspartic acid, glutamyl, aspartyl containing peptides, nucleotides (inosine-5'-monophosphate, guanosine-5'-monophosphate)- by combining a -preferably soy and wheat free- substrate bed from a) a first amount of one or more wet ingredients, having a first moisture content which preferably is between 60% and 95% by weight, more preferably between 70% and 90% by weight, most preferably between 80% and 85% by weight, and b) a second amount one or more dry water-absorbing unboiled unsteamed ingredients, having a second moisture content that is smaller than the first moisture content and preferably between 1% and 30% by weight, more preferably between 5% and 20% by weight, most preferably 7% and 15% by weight, wherein the ratio between the first amount and the second amount is selected to result in the overall moisture content of the substrate bed to be between 39% and 63% by weight, preferably between 45% and 59% by weight;

- inoculating the multi-component substrate system with filamentous fungi;

- carrying out solid-state fermentation leading to hydrolysis and mycelial growth to convert the substrate bed to fermented umami-containing enzymatically active biomass.

2. The method according to claim 1, **wherein:** the dry ingredients are chosen to form a mixture of larger particles for aeration of the multi-component substrate system and smaller particles for absorbing water from the wet ingredients.

3. The method according to claim 2, **wherein:** the dry ingredients are selected based on their particle size.

4. The method according to any one of the preceding claims 1 - 3, **wherein:** the water distribution within the multi-component substrate system is controlled with water holding capacity of the dry ingredients, most advantageously by mixing, which preferably is carried out with a sufficiently low power or speed to avoid grinding of the ingredients that causes agglomeration of the substrate bed.

5. The method according to any one of the preceding claims 2 – 4, **wherein:** between 0,5% and 40% by weight, preferably between 1% and 20% by weight, of the particles in the dry ingredients have a size larger than 4 mm.

6. The method according to any one of the preceding claims 2 - 5, **wherein:** the dry ingredients are selected to contain 20 – 70%, preferably 30 – 60%, of particles having a diameter in the range of 0,75 to 3 mm.

7. The method according to any one of the preceding claims 1 - 6, **wherein:** in the method, natural hydration properties i.e. water holding capacity of the ingredients of the substrate bed are controlled.

8. The method according to claim 7, **wherein:** the controlling is carried out by i) adjusting the particle size and ratio of dry/wet ingredients to control water holding capacity and/or ii) indirectly such that water holding capacity is increased by increasing share of non-starch polysaccharide containing ingredients.

9. The method according to any one of the preceding claims 7 or 8, **wherein:** the dry water-absorbing ingredients are selected to have a water holding capacity of at least 100% of their weight, and/or a part, preferably at least 20% of dry ingredients, has a water holding capacity of at least 150% of its weight.

10. The method according to any one of the preceding claims 1 - 9, **wherein:** dry ingredients are heated, preferably to above 85°C, to reduce microbial load, before combining with wet ingredients and inoculation with fungal spores or mycelia; and/or the substrate bed is heated -preferably to above 75°C or to above 85°C and/or preferably by steaming- upon the wet ingredients have been combined

-preferably by mixing- with the dry water-absorbing unboiled unsteamed ingredients, to reduce microbial load, after which the substrate bed is allowed to cool before it is inoculated with fungal spores or mycelia.

11. The method according to any one of the preceding claims 1 - 10 **wherein:** wet ingredients are heated in dry heat, preferably to above 75°C, to reduce microbial load, before combining with dry ingredients and inoculation with fungal spores or mycelia; and/or

the substrate bed is heated -preferably to above 75°C or to above 85°C and/or preferably by steaming- upon the wet ingredients have been combined -preferably by mixing- with the dry water-absorbing unboiled unsteamed ingredients, to reduce microbial load, after which the substrate bed is allowed to cool before it is inoculated with fungal spores or mycelia.

12. The method according to any one of the preceding claims 1 - 11, **wherein:** the combined ingredients are considered to be suitable for producing umami amino acids if:

i) the combined ingredients comprise at least 1400 mg/100 g glutamic acid,

ii) optionally, if in addition to i), the combined ingredients further comprise at least 18% by weight carbohydrates;

iii) optionally, if in addition to i) and preferably also to ii), the pH of the combined ingredients is in the range of 4,8 - 6,8.

13. The method according to any one of the preceding claims 1 to 12, **wherein:** the substrate bed is fermented at 26 - 40 ℃, preferably 26 - 36 ℃, at relative humidity between 60 and 100%, preferably between 80 and 100%, for 12-72h, preferably 15-24 hours, preferably under ventilation and/or movement to prevent the substrate bed from overheating.

14. The method according to claim 13, **wherein:** the internal temperature of the substrate bed is, after the inoculation, kept

below 40°C, which is preferably ensured by turning the substrate bed periodically.

15. The method according to any one of the preceding claims 1 - 14, **wherein:** the solid-state fermentation is tested for success a) by controlling the extent of proteolysis quantified as i) increase in primary amino nitrogen, ii) decrease of residual protein, iii) release of glutamic acid/glutamate and/or by b) testing for the release of reducing sugars from hydrolysis of starch and non-starch polysaccharides.

16. The method according to any one of the preceding claims 1 to 15, **wherein:** the particle size distributions are controlled to have aerated conditions for at least one aerobic fungus used in the solid state fermentation to increase enzyme activities for an improved flavor and flavor precursor production.

17. The method according to any one of the preceding claim 16, **wherein:** in the particle size distribution, the surface area to volume ratio is controlled.

18. The method according to any one of the preceding claims 1 - 17, **wherein:** the unboiled unsteamed dry ingredients in the substrate bed are natural ingredients for which no such cooking step is performed which substantially increases the moisture in the ingredient and/or which substantially reduces the water absorption capacity of the ingredient.

19. The method according to any one of the preceding claims 1 – 18, **wherein:** the wet ingredients are derived from wet side streams from vegetable, legume, oilseed and/or cereal processing industries, such as fresh or frozen vegetable processing side streams, brewer’s spent grain, plant milk or protein extraction by-products, broken legumes and beans, oil seed press cakes, bread and dough waste, and rejected fruits and vegetables, vegetable and fruit processing by-products such as peels or rejected pieces, legume processing side streams with high moisture content such as high moisture extrusion waste.

20. The method according to any one of the preceding claims 1 - 19, **wherein:** the dry ingredients comprise at least one of the following:

underutilised milling fractions such as hulls, husk, seed coat, and bran.

21. The method according to any one of the preceding claims 1 to 20, **wherein:** the dry and/or wet ingredients include at least one of the following:

i) non-soy legumes, such as pea, faba bean, lupin, lentil, chickpeas, brown beans;

ii) grains, such as oat, barley, rye, buckwheat, wheat quinoa, millet;

iii) combinations of different legumes from i) and/or grains from ii);

iv) dry legume processing side stream, preferably from milling, classification, extrusion processes;

v) fresh legume and/or vegetable processing side streams, such as classification waste from frozen pea or bean production, preferably including split or otherwise damaged fresh peas and their hulls;

vi) oilseed processing waste, such as hemp, rapeseed, sunflower, olive press cakes;

vii) other seed processing waste, such as press cakes from production of extracts;

viii) food waste, such as bread and other cereal foods, processed vegetable or legumes containing foods.

22. The method according to any one of the preceding claims 1 to 21, **wherein:** the dry and/or wet ingredients include side stream from one, two, three or more of the following:

- fresh pea processing, preferably damaged fresh green peas;
- dried peas, preferably whole, cracked or milled;
- dried faba beans, preferably whole, cracked or milled;
- cereal flours or flakes;

- milling side streams;

- bakery side streams;

- side stream from plant protein extrusion.

23. The method according to any one of the preceding claims 1 to 22, **wherein:** in the solid state fermentation, fungal genera used for include at least one non-toxic species of the following *Aspergillus, Rhizopus, Geotrichium, Penicillium, Lentinula, Pleurotus, Auricularia, Agaricus, Flammulina, Hericium, Clitocybe, Hypsizygus, Sparassis, Ustilago, Fusarium*; preferably, at least <*Aspergillus Oryzae* is used.

24. The method according to any one of the preceding claims 1 to 23, **wherein:** the fermented umami-containing enzymatically active biomass is further processed into flavor products.

25. The method according to claim 24, **wherein:** the processing into flavour products is carried out using a high ionic strength ripening step.

26. The method according to claim 24, **wherein:** the processing into flavour products is carried out using a low ionic strength ripening step.

27. A method of producing an umami concentrate, **wherein:**

- fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to any one of the preceding claims 1 to 26, is mixed with 8 - 20%, preferably 10-18%, salt aqueous solution and the resulting mash is ripened in liquid state in the presence of halophilic bacteria and yeasts, preferably for 1 to 24 months, preferably at ambient temperature such as 15-25 ℃;

- after ripening, the mash is pasteurised and insoluble dry matter is removed, such as by pressing, decanting, centrifuging, or filtering, to produce an umami concentrate.

28. The method according to claim 27, **wherein:** the umami concentrate is packaged to be used as such or used in the formulation of seasoning products.

29. A method for producing an umami paste, **wherein:**

- fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to any one of the preceding claims 1 to 26, is mixed with salt and unfermented legumes, cereal products and/or vegetables,

- optionally blended the mixture to produce a paste

- ripening in the presence of halophilic bacteria and yeasts, such as for 1 month to 8 months, preferably at ambient temperature, such as 15-22℃.

30. The method according to claim 29, **wherein:** the ripened product is pasteurized and formulated into seasoning products.

31. A method for producing solid umami products, **wherein** at least one of the following steps is carried out:

a. umami-containing concentrate or extract produced by ripening fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to any one of the preceding claims 1 to 26, is dried into powder.

b. fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to any one of the preceding claims 1 to 26, is used to formulate a solid, gratable seasoning or food product that is optionally solidified, preferably using starches, fats and/or hydrocolloids.

c. fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to any one of the preceding claims 1 to 26, is dried into powder or chunks.

d. fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to any one of the preceding claims 1 to 26 is used to formulate a solid, gratable seasoning and/or food product that optionally is solidified, preferably using starches, fats and/or hydrocolloids.

32. A method for producing salt-free or low-salt umami extract, **wherein:**

- umami extract is produced by mixing fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, preferably produced with a method according to any one of the preceding claims 1 to 26, with water to produce a slurry;

- optionally adding endogenous enzymes;

- mashing the slurry so that it undergoes enzymatic processing; and

- pasteurizing and filtering the slurry.

33. The method according to claim 32, **wherein:** the mashing is carried out for 3 to 5 h.

34. The method according to claim 32, **wherein:** the mashing is carried out for more than 5 h, but preferably less than 24 h.

34. The method according to any one of the preceding claims 32 - 34, **wherein:** the mash is acidified, preferably using lactic acid bacteria.

35. The method according to any one of the preceding claims 32 – 34, **wherein:** the filtered slurry is used as such as liquid seasoning product, dried, concentrated or mixed with other ingredients.

36. A method for producing a low-salt umami product, **wherein:** a salt free or low-salt umami extract preferably produced with the method according to any one of the claims 32 to 35, is combined with an umami concentrate preferably produced with the method according to claim 27 or 28.

37. The method according to claim 36, **wherein:** the adjusting ratio between the salt free or low-salt umami extract and the umami concentrate, the salt content, color and flavor are controlled.

38. The method according to claim 36 or 37, **wherein:** flavor modulators -such as vegetables, fruits, herbs, mushrooms, algae- are added before cooking to modify flavor profile.

39. The method according to 38, **wherein:** the low salt umami product is a liquid that with the flavor modulators is altered from neutral to resemble meat, chicken, fish or bone broth.

40. The method according to any one of claims 36 to 39, **wherein:** the resulting product is used as base for stocks or as neutral flavorings for dishes and food products.

41. A fermented umami-containing enzymatically active -preferably soy and wheat free- biomass, **characterized in that:** it has been manufactured with the method of any one of claims 1 to 26.

42. An umami concentrate -that is preferably soy and wheat free-, **characterized in that:** it has been manufactured with the method of claims 27 or 28.

43. An umami paste -that is preferably soy and wheat free-, **characterized in that:** it has been manufactured with the method of claims 29 or 30.

44. A solid umami product -that is preferably soy and wheat free-, **characterized in that:** it has been manufactured with the method of claim 31.

45. A salt-free or low-salt umami extract -that is preferably soy and wheat free-, **characterized in that:** it has been manufactured with the method of any one of claims 32 to 35.

46. A low-salt umami product -that is preferably soy and wheat free-, **characterized in that:** it has been manufactured with the method of any one of claims 36 to 40.

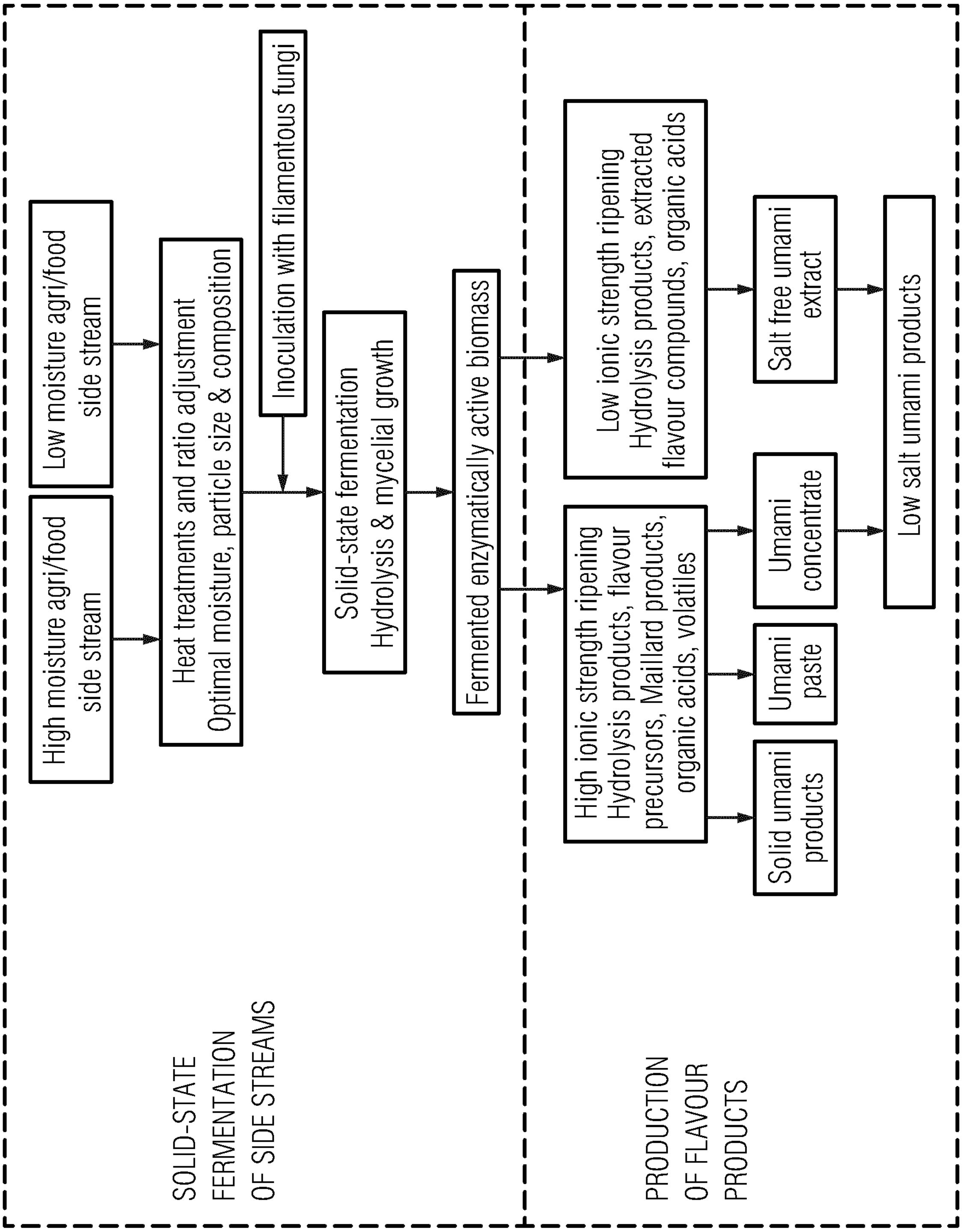

SOLID-STATE FERMENTATION OF SIDE STREAMS
High moisture agri/food side stream
Low moisture agri/food side stream
Heat treatments and ratio adjustment
Optimal moisture, particle size & composition
Inoculation with filamentous fungi
Solid-state fermentation
Hydrolysis & mycelial growth
Fermented enzymatically active biomass
PRODUCTION OF FLAVOUR PRODUCTS
High ionic strength ripening
Hydrolysis products, flavour precursors, Maillard products, organic acids, volatiles
Low ionic strength ripening
Hydrolysis products, extracted flavour compounds, organic acids
Solid umami products
Umami paste
Umami concentrate
Salt free umami extract
Low salt umami products

FIG 1

FIG 2

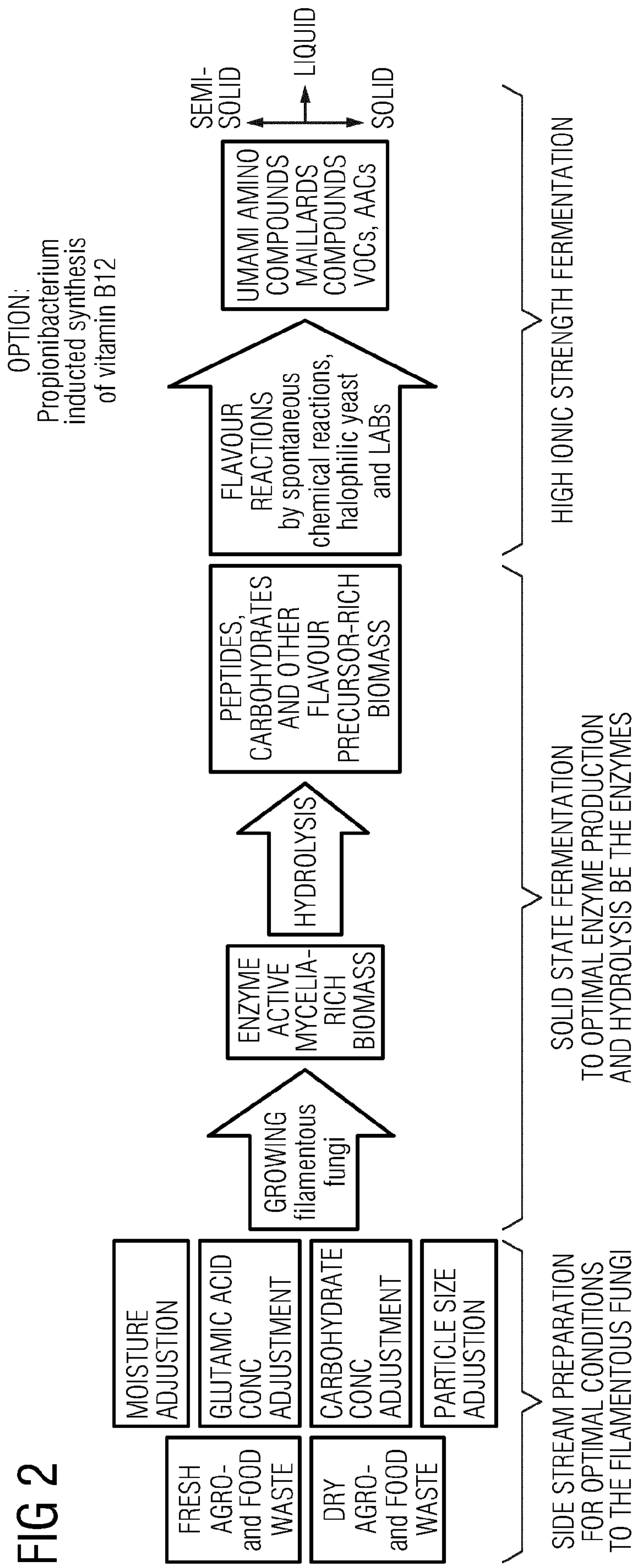
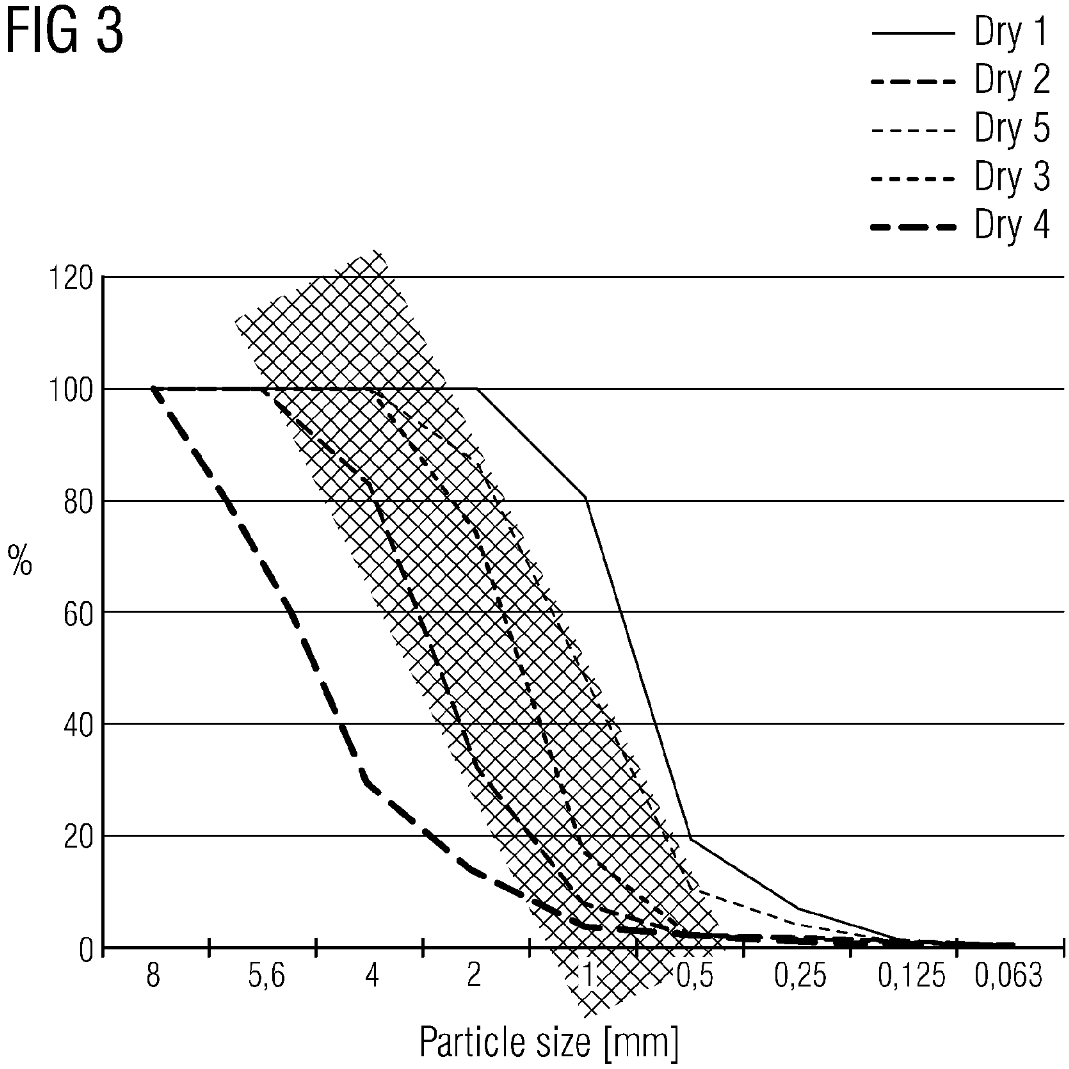

FRESH AGRO- and FOOD WASTE
DRY AGRO- and FOOD WASTE
MOISTURE ADJUSTION
GLUTAMIC ACID CONC ADJUSTMENT
CARBOHYDRATE CONC ADJUSTMENT
PARTICLE SIZE ADJUSTION
GROWING filamentous fungi
ENZYME ACTIVE MYCELIA-RICH BIOMASS
HYDROLYSIS
PEPTIDES, CARBOHYDRATES AND OTHER FLAVOUR PRECURSOR-RICH BIOMASS
FLAVOUR REACTIONS by spontaneous chemical reactions, halophilic yeast and LABs
OPTION: Propionibacterium inducted synthesis of vitamin B12
UMAMI AMINO COMPOUNDS MAILLARDS COMPOUNDS VOCs, AACs
SEMI-SOLID
LIQUID
SOLID
SIDE STREAM PREPARATION FOR OPTIMAL CONDITIONS TO THE FILAMENTOUS FUNGI
SOLID STATE FERMENTATION TO OPTIMAL ENZYME PRODUCTION AND HYDROLYSIS BE THE ENZYMES
HIGH IONIC STRENGTH FERMENTATION

FIG 3


Dry 1
Dry 2
Dry 5
Dry 3
Dry 4
120
100
80
60
40
20
0
%
8
5,6
4
2
1
0,5
0,25
0,125
0,063
Particle size [mm]

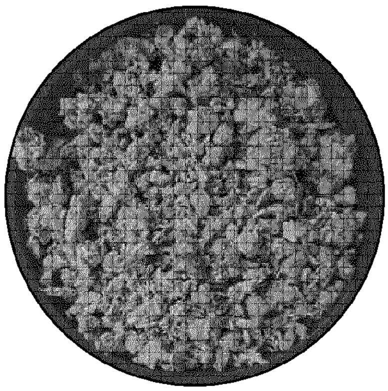


FIG 4C

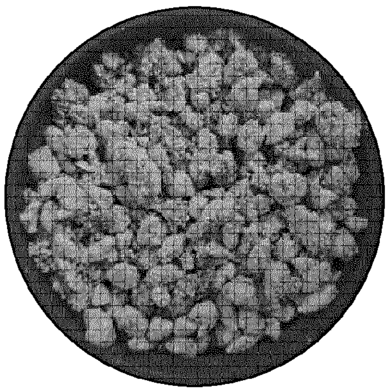


FIG 4B

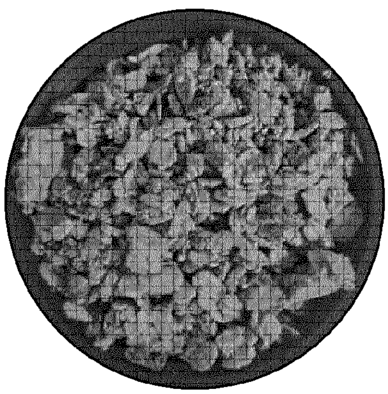


FIG 4A

FIG 5

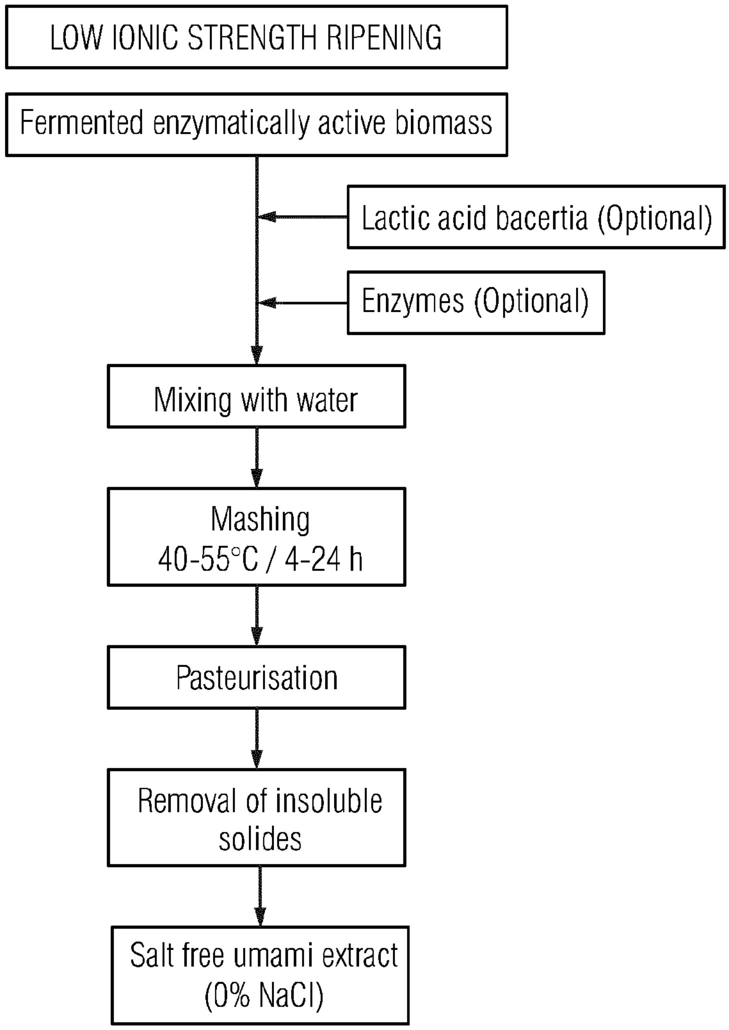

LOW IONIC STRENGTH RIPENING
Fermented enzymatically active biomass
Lactic acid bacertia (Optional)
Enzymes (Optional)
Mixing with water
Mashing
40-55°C / 4-24 h
Pasteurisation
Removal of insoluble solides
Salt free umami extract
(0% NaCl)

FIG 6

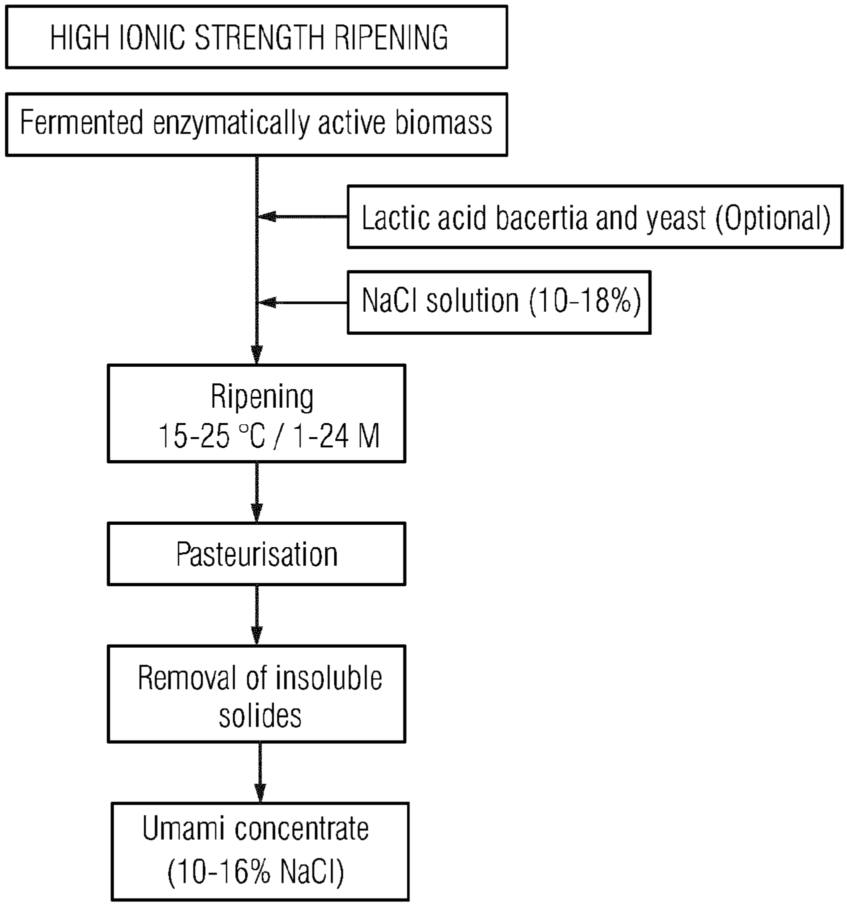

HIGH IONIC STRENGTH RIPENING
Fermented enzymatically active biomass
Lactic acid bacertia and yeast (Optional)
NaCl solution (10-18%)
Ripening
15-25 °C / 1-24 M
Pasteurisation
Removal of insoluble solides
Umami concentrate
(10-16% NaCl)

FIG 7

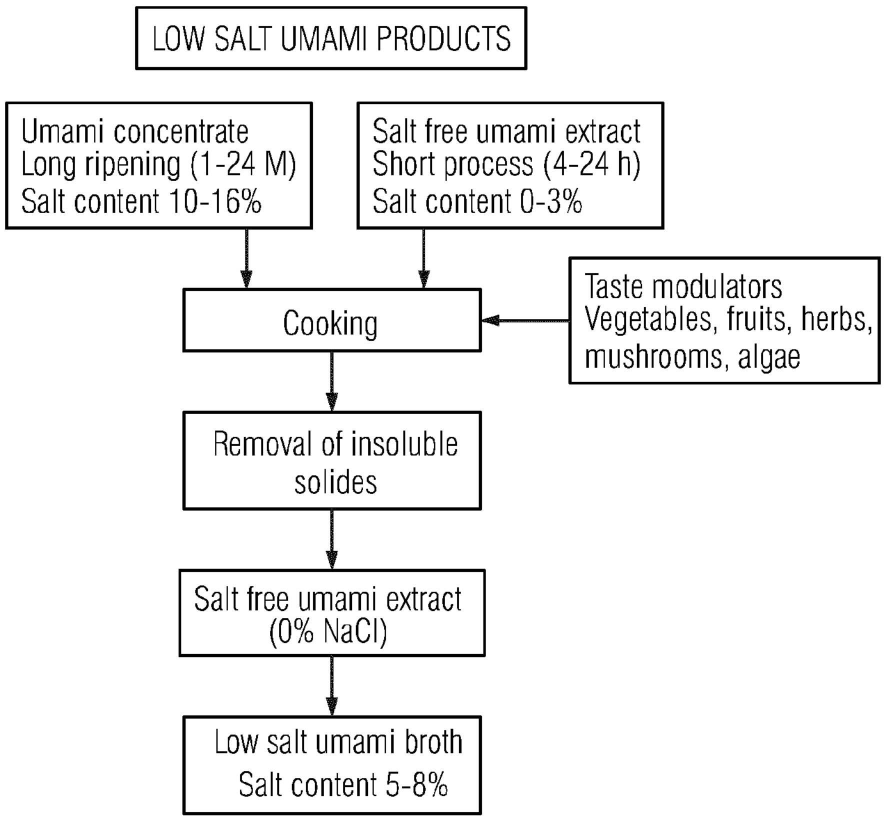

LOW SALT UMAMI PRODUCTS
Umami concentrate
Long ripening (1-24 M)
Salt content 10-16%
Salt free umami extract
Short process (4-24 h)
Salt content 0-3%
Taste modulators
Vegetables, fruits, herbs,
mushrooms, algae
Cooking
Removal of insoluble
solides
Salt free umami extract
(0% NaCl)
Low salt umami broth
Salt content 5-8%

FIG 8

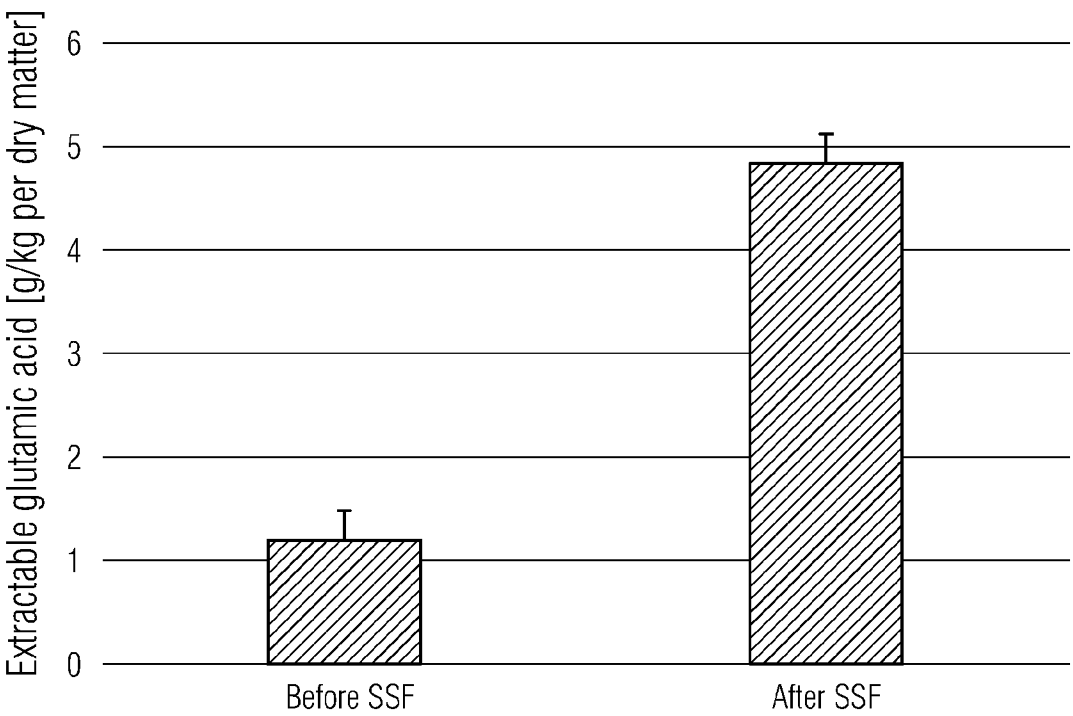

Extractable glutamic acid [g/kg per dry matter]
6
5
4
3
2
1
0
Before SSF
After SSF

FIG 9

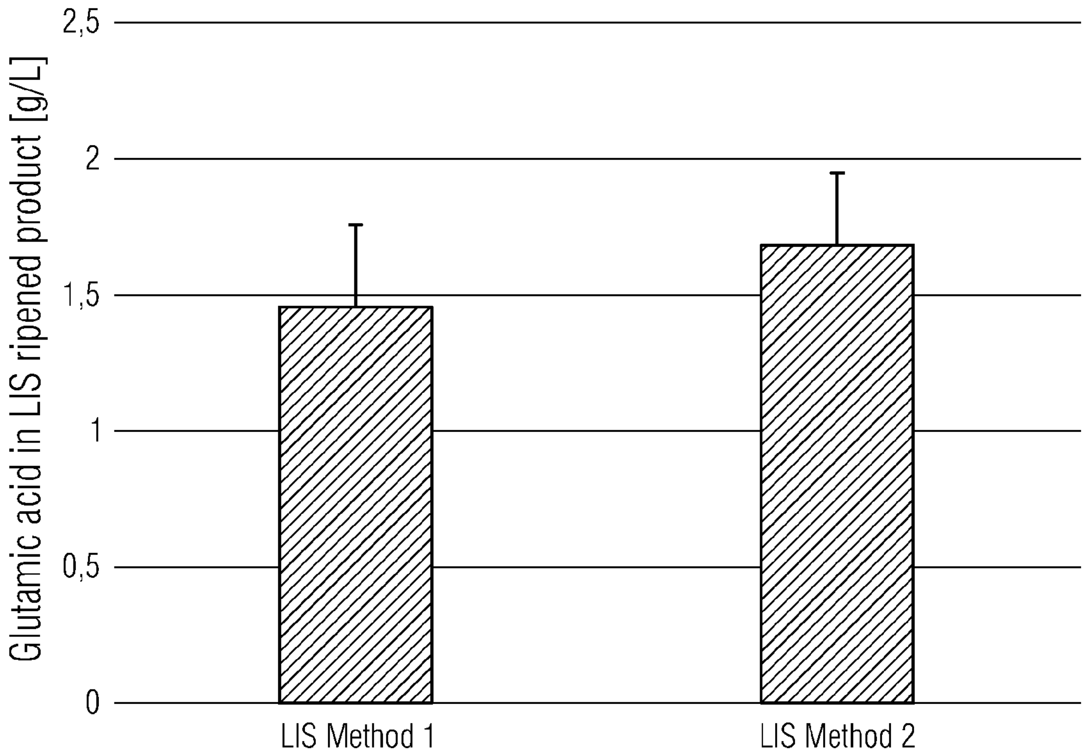
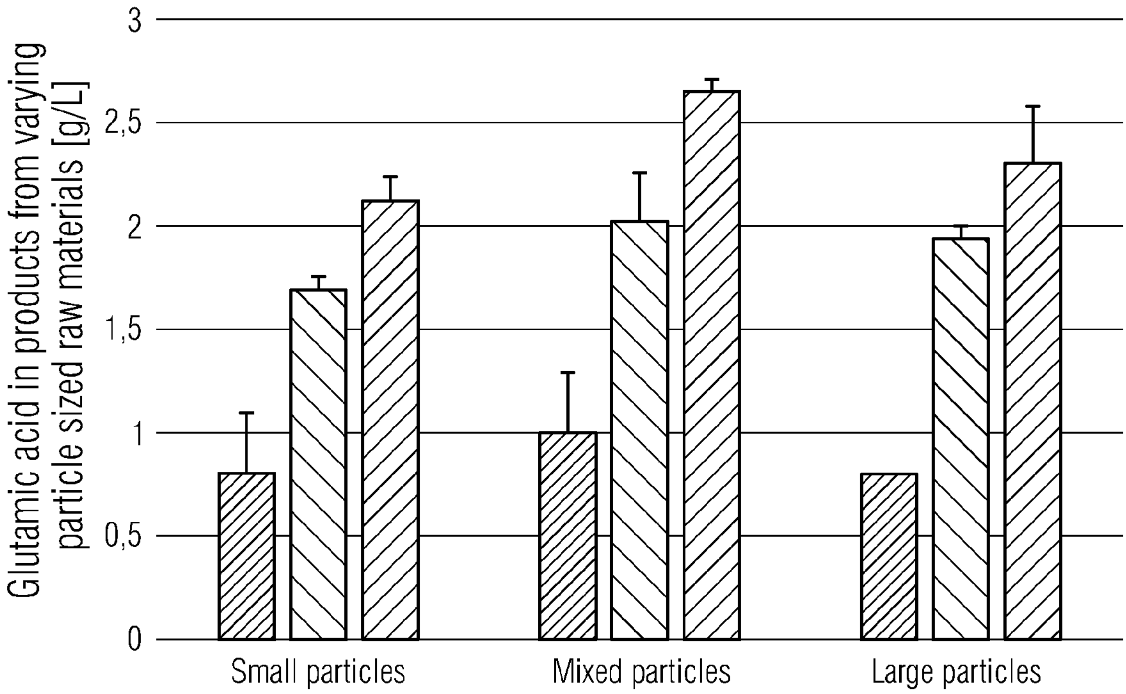

2,5
2
1,5
1
0,5
0
Glutamic acid in LIS ripened product [g/L]
LIS Method 1
LIS Method 2

FIG 10


Glutamic acid in products from varying particle sized raw materials [g/L]
3
2,5
2
1,5
1
0,5
0
Small particles
Mixed particles
Large particles

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/060864

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12P1/02 A23L27/00 A23L31/15 A23L33/185 C12N1/14
C12P13/14 C12P13/20 C12P19/30 A23L27/40 A23L27/22
A23L27/23 A23L27/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12P A23L C12R C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	EP 2 835 058 A1 (STICHTING ECO CONSULT [NL]) 11 February 2015 (2015-02-11) page 1, paragraphs [0001],[0010],[0013] page 2, paragraph [0016]-[0020]; examples 1-3	1,31,41, 44 2-30, 32-40
X Y	CN 100 405 922 C (RONGGAO SUN [CN]) 30 July 2008 (2008-07-30) the whole document	41,44 2-30, 32-40
X Y	WO 2020/232347 A1 (MYCOTECHNOLOGY INC [US]) 19 November 2020 (2020-11-19) page 1, paragraph [0008] - page 2, paragraph [0010]; examples 1-17 claims 1-24	41,44 2-30, 32-40

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 July 2023	08/08/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Mateo Rosell, A

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2021/337827 A1 (WHITELEY JUSTIN [US] ET AL) 4 November 2021 (2021-11-04) page 1, paragraphs [0002], [0005]-[0006] page 2, paragraph [0017] page 3, paragraph [0022]-[0025] page 5, paragraph [0051] - page 6, paragraph [0060] -----	41,44
X	Anonymous: "Fava Bean Umami Paste (Fermented beans full of rich umami flavour!)", Wholesome Weigh, 22 September 2021 (2021-09-22), XP093047455, Retrieved from the Internet: URL:https://web.archive.org/web/20210922072851/http://wholesomeweigh.co.uk/product/fava-bean-umami-paste-fermented-beans-full-of-rich-umami-flavour/ [retrieved on 2023-05-16] the whole document -----	42-44
X	US 2014/065131 A1 (KELLY BROOKS JOHN [US] ET AL) 6 March 2014 (2014-03-06) page 1, paragraph [0012] page 2, paragraph [0013] - page 3, paragraph [0034] page 3, paragraph [0071]-[0072] pages 4-5, paragraph [0082] -----	45
X	JP 2017 143767 A (KIKKOMAN CORP) 24 August 2017 (2017-08-24) the whole document -----	46
X	US 2014/004225 A1 (AKAMATSU HIROYUKI [JP] ET AL) 2 January 2014 (2014-01-02) page 1, paragraph [0001] page 2, paragraph [0018]-[0024]; example 3; table 1 page 3, paragraph [0033]-[0042] page 5, paragraph [0058]-[0078] examples 1-3 -----	46

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2023/060864

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 2835058	A1	11-02-2015	EP 2835058	A1	11-02-2015
			NL 2011277	C2	10-02-2015
CN 100405922	C	30-07-2008	NONE		
WO 2020232347	A1	19-11-2020	CA 3139907	A1	19-11-2020
			EP 3968776	A1	23-03-2022
			US 2022232854	A1	28-07-2022
			WO 2020232347	A1	19-11-2020
US 2021337827	A1	04-11-2021	NONE		
US 2014065131	A1	06-03-2014	US 2014065131	A1	06-03-2014
			US 2014065263	A1	06-03-2014
JP 2017143767	A	24-08-2017	NONE		
US 2014004225	A1	02-01-2014	JP 5968304	B2	10-08-2016
			JP WO2012128290	A1	24-07-2014
			TW 201244641	A	16-11-2012
			US 2014004225	A1	02-01-2014
			WO 2012128290	A1	27-09-2012