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(54) Benævnelse: **FREMGANGSMÅDE TIL FREMSTILLING AF ET FØDEVAREMIDDEL ELLER EN FORLØBER DERAF, FØDEVAREMIDDEL ELLER EN FORLØBER DERAF OG PASSENDE ANVENDELSER.**

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Description

The present invention relates to a method for the preparation of a food or a precursor of the same according to claim 1, a food or a precursor of the same according to claim 9 as well as the uses according to claims 10 and 11.

Vitamin B_{12} plays a significant role in cell division, blood formation, functioning of the nervous system, in mental faculties as well as in the metabolism of carbohydrates, fats and proteins. A deficiency of vitamin B_{12} may lead to serious metabolic disorders or other physical impairments, such as e.g. a reduced energy metabolism or an impaired immune system.

An adequate supply of the human or animal body with vitamin B_{12} therefore represents an important prerequisite for health and fitness.

Definitions

According to the invention the terms, "foodstuff" or "food" are understood to mean all substances and products that the person skilled in the art understands hereunder. Thus, "foodstuff" can include all substances and products that are suitable for human and/or animal consumption and that preferably exhibit a nourishing effect. In particular, in the context of this invention the term, "foodstuff" can include: A cereal-based foodstuff, a cereal-based bar, a malt extract product, breakfast cereals, a pastry, a dairy product, a yoghurt, a drink, an alcohol-free drink, a beer, an alcohol-free beer, a beer brewed from wheat, an alcohol-free beer brewed from wheat, a mixed beer drink, a drink fermented with yeast, a drink not fermented with yeast and/or concentrates of the abovementioned foodstuffs. A precursor of the foodstuff according to the invention, in particular of the drink, can be in particular an acid malt, a mash and a wort. In the context of this Application the term, "non-fluid" relating to foodstuffs, includes a solid, pasty, paste-like, jelly-like or similar consistency of a foodstuff.

Vitamin B_{12} is a collective term for a series of water-soluble, structurally similar compounds with a biological action, the corrinoids. Due to the complexly bonded cobalt atom they are called cobalamines. Methylcobalamin,

desoxyadenosylcobalamin, hydroxycobalamin and sulfitocobalamin are regarded by the person skilled in the art as biologically active forms of vitamin B_{12}. Of these, methylcobalamin and desoxyadenosylcobalamin are considered to be the most biologically effective or most active forms. Of this group, cyanocobalamin may only be obtained synthetically.

In addition to these active vitamin B_{12} forms there are also “inactive analogues”. These are also called pseudo vitamin B_{12}, because although they have a similar chemical structure as the real vitamin B_{12}, under certain circumstances, however, they do not develop any vitamin action for the human or animal body. They are unable to fulfil the physiological functions of vitamin B_{12} in the organism. Moreover, they may block the uptake and metabolisation of the biologically active vitamin B_{12}.

In the context of this Application pseudo vitamin B_{12} is especially understood to mean 7-adeninyl-cyanocobamides.

In the context of this Application the definition of the term, “vitamin B_{12}” is limited to the forms of vitamin B_{12} or its precursors that are active in the human or animal organism. Furthermore, the term, “vitamin B_{12}” in the context of this Application is limited to the forms of vitamin B_{12} that can be produced microbiologically. As the abovementioned cyanocobalamin may only be produced synthetically, and is therefore not a "natural” vitamin B_{12}, this form of vitamin B_{12} is not included in the term, “vitamin B_{12}" in the context of this Application.

Consequently, in the context of the present Application, the definition of the term, “vitamin B_{12}” is limited to the following vitamin B_{12} species: methylcobalamin, deoxyadenosylcobalamin, hydroxocobalamin and sulfitocobalamin.

The members of the above cited group are generally characterised by high bioavailability, such that they are able to improve the supply of vitamin B_{12} to the human or animal body.

In the context of the present Application, the term, “bioavailability” is understood to mean the resorption of the foodstuff (vitamin B_{12}) from the gastrointestinal tract into the blood, whereby the blood reaches the systemic circulation and

thereby becomes available to the cells/organs. Furthermore, for the definition of the terms, “bioavailable” or “bioavailability”, reference may be made to paragraph [0013] of the description of the Internationalen Patent-Offenlegungsschrift WO 2012/109 324 A1. Explicit reference is hereby made to the entire last document.

The bioavailable vitamin B_{12} is determined analytically by means of the ADVIA system. This bioavailability determination is based on the “Intrinsic Factor” (IF) and is used for example to determine bioavailable vitamin B_{12} in the blood. Vitamin B_{12} is absorbed in the last intestinal segment (Ileum) by means of the Intrinsic Factor (IF = protein that is secreted into the parietal cells in the gastric mucous membrane). In the context of these tests the IF-unbound vitamin B_{12} is defined as non-bioavailable, because it does not reach the blood circulation and concomitantly does not attain the corresponding tissue.

The ADVIA Centaur VB12-Test (111659 Rev. N, 2008-09; VB12 2/12) was used in the present Application to determine the bioavailable vitamin B_{12}. This is a competitive immunoassay involving direct chemiluminescence technology. In this regard the vitamin B_{12} in the sample competes with the vitamin B_{12} marked with acridinium ester in a Lite reagent for a limited amount of purified IF that is covalently bonded on paramagnetic particles in the solid phase. In this test the release agent (sodium hydroxide) and DTT are used to release the vitamin B_{12} from the endogenous binding proteins in the sample. Added cobinamide prevents rebinding after the addition of the solid phase to the sample.

The (total) concentration of vitamin B_{12}, including all forms of bioavailable and non-biologically available forms of vitamin B_{12}, is determined in the context of this Application by the use of the measurement method r-Biopharm AOAC-Methode Nr. 101002. Alternatively, the total concentration can be measured by "Fresenius AOAC-Methode Nr. 952. 20".

In relation to a yeast product, the term, “autolysate” is understood according to the invention to mean a mostly fluid nutritive medium that is obtained by dissolving the cells of baker’s yeast, feed yeast (protein yeast) and/or especially beer yeast by cellular enzymes.

In relation to a yeast product the term, “extract” is inventively understood to mean a mostly powdered, gel-like or pasty product from a yeast autolysate (produced by means of internal yeast enzymes) or yeast hydrolysate (produced by means of external enzymes) with a high content per dry mass of amino acids (e.g. 30 to 50 %), carbohydrates (e.g. 20 to 30 %) and vitamins (especially: B-group: thiamine, riboflavin, nicotinic acid). Generally, a yeast extract is produced by at least partially freeing a yeast autolysate or a yeast hydrolysate from the insoluble cell components, concentrating it and optionally spray drying it. A yeast extract typically has a dry substance content of 70 to 80 % in a pasty form and from 95 to 97 % in a powdered form.

In relation to a yeast extract, the term, “dried form" is inventively understood to mean each form of a yeast having a water content of maximum 5 %, preferably maximum 2 %, especially maximum 1 %. Hereinafter a dried yeast is understood in particular to mean a yeast in the form of flakes or powder or a freeze-dried yeast.

In the context of this invention the definition commonly used in the field of brewing technology is assigned to the term, “acid malt” (Sauergut). In particular, this is understood to mean a nutrient medium, in particular a mash and/or wort, treated microbially to lower the pH, preferably with lactic acid bacteria.

In the context of this Application the terms "mash”, “wort” and “last runnings” are understood to mean in particular the substrates known to the person skilled in the art as designated in the beer preparation process. However, the terms are inventively not necessarily limited to them; they may also relate to analogous substrates, i.e. precursors, in regard to other drinks or other foodstuffs, such as for example whisky mashes. In particular, the wort may be inventively produced from a mash according to the invention, as defined herein.

The term, “mash” can be inventively preferably limited to a mash that was produced by using a carbohydrate-containing substrate or a mixture of carbohydrate-containing substrates. In this regard the carbohydrate-containing substrate or the mixture of carbohydrate-containing substrates possesses a fraction of brewing malt of at least 80 wt %, preferably at least 90 wt %, preferably at least 95 wt %, preferably at least 98 wt %, preferably at least 99 wt %, in particular about 100 wt %.

In particular, the term, "mash" can be inventively limited to a mash that was produced by using brewing malt, wherein the brewing malt possesses a fraction of wheat malt of at least 50 wt %, preferably at least 52 wt %, in particular at least 55 wt %. The term, "brewing malt" inventively includes malts of one variety and also mixtures of various malt varieties.

Furthermore, the term, "wort" can be inventively limited to a wort that was obtained from a mash that was produced by using brewing malt with a fraction of wheat malt of at least 50 wt %, preferably at least 52 wt %, in particular at least 55 wt %.

In the context of this Application the term, "hop bitter substances" is understood to mean all the bitter substances from hops and hops resins, which are known to the person skilled in the art. These include both the soft resins a well as the hard resins, including the bitter acids, in particular the α-acids and β-acids, and the known derivatives of these resins and acids, in particular their oxidation products.

In the context of this Application the term, "free from hop bitter substances" relating to a medium (e.g. nutrient medium or yeast product) is understood to mean the complete absence of hop bitter substances in this medium.

The term, "essentially free of hop bitter substances" relating to a first nutrient medium is understood in the context of this Application to mean a content of hop bitter substances of maximum 15 %, preferably maximum 10 %, preferably maximum 5 %, especially maximum 2 %, based on the content of hop bitter substances which a typical brewery wort has for a fermentation with a bottom-fermented or top-fermented yeast with bitter units (EBC Method) in the range 15 to 38, preferably 20 to 35. In this regard the abovementioned percentages may also be inventively applied to each individual substance of the group of the hop bitter substances (e.g. humulon or lupulon).

The term, "essentially free of hop bitter substances" relating to a yeast product is understood in the context of this Application to mean a content of hop bitter substances of maximum 20 %, preferably maximum 15 %, preferably maximum 10 %, preferably maximum 5 %, especially maximum 2 %, based on the content of hop bitter substances which a typical brewery yeast has for a fermentation with a bottom-fermented or top-fermented yeast, in particular a fresh yeast harvested

from the first, second or third run, which during a fermentation of a typical brewery wort was fermented with bitter units (EBC Method) in the range 15 to 38, preferably 20 to 35. In this regard the abovementioned percentages may also be inventively applied to each individual substance of the group of the hop bitter substances (e.g. humulon or lupulon).

Analogously to the previous definition, the term, "hop bitter substances ... completely or essentially completely removed ..." in the context of the present Application is also understood to relate to a yeast or a yeast product.

The subject matter of the invention can relate to the production of alcoholic and non-alcoholic drinks in the brewery, especially beer, and the corresponding products. In the context of this Application the terms, "first wort", "boiling", "keeping warm", "lees", "pitching temperature", "pure selected yeast", "harvested yeast", "washing the yeast", "processing to a drink", "wort acidification", "mash acidification" etc. are then each attributed the meaning that a person skilled in the art usually attributes to the technical term or the activity commonly used in the field of drink production, especially in the field of beer production. The same applies to the use of terms for objects or activities within this Application which relate to the production or treatment of solid or pasty or gel-like foodstuffs and the corresponding products.

In the context of this Application "treatment" or "treating" with lactic acid bacteria or yeast is understood to mean each kind of partial or complete metabolisation of nutrients from a nutrient medium, in particular a partial or complete fermentation by lactic acid bacteria. Moreover, "treating" a nutrient medium can be each kind of bringing into contact or being in contact with microorganisms, preferably lactic acid bacteria or yeast, in particular with the lactic acid bacteria and beer yeasts inventively provided in this Application.

In the sense of this Application the term, "lactic acid bacteria" includes each species and state of lactic acid bacteria, i.e. any type or sub-species or any strain, insofar as it has not been further limited in this Application. This term further includes living and/or dead bacterial cells, especially living bacterial cells. The DSMZ numbers given in this Application serve only to clearly identify the inventively used bacteria species or sub-species. However, the invention is not

limited to the DSMZ as the sole reference source for these inventively used bacteria species or sub-species.

"Wheat beer" is inventively understood to mean a top-fermented beer with a wheat malt content of at least 50 wt %, preferably at least 52 wt %, especially at least 55 wt % in the bulk or in the carbohydrate-containing substrate.

The term, "alcohol-free" relating to a foodstuff, drink or beer or to another product named in this application is inventively understood to mean an ethanol content in the product of 0 to less than 1.2 vol. %, preferably 0 to less than 1.0 vol. %, preferably 0 to less than 0.5 vol. %, preferably 0 to less than 0.3 vol. %, preferably 0 to less than 0.1 vol. %, especially about 0 volume %.

In the context of this Application the statements "about", "approximately" or the like are understood to mean a relative deviation from the stated value of at most 10 %, preferably at most 5 %, preferably at most 3 %, in particular at most 1 %.

The volume or volume fraction data used in this Application always refer to the temperature or temperatures that a person skilled in the art would typically attribute to the fluid or mixture used for each purpose or for each process step, insofar as a temperature is not explicitly stated.

All weights and weight concentrations stated in the Application refer to the dry weight of the substance in question.

Prior art

The document EP 0 545 139 B1 discloses a malt drink that has an unfermented malt brew with an original wort content between 2 and 15 %. This malt drink can be slightly hopped and has a cyanocobalamin content of 5 to 30 microgram per litre.

In the document EP 824 152 B1 is described the production of the natural vitamin B_{12} in relatively high concentrations (≥ 0.1 wt %) by using *Propionibacterium freudenreichii.*

Similarly, GB 793 467 A relates to the production of preparations that have a high vitamin B_{12} activity, in particular by cultivating a certain type of *Propionibacterium,* in particular *P. freudenreichii* or *P. shermanii.* A method for

producing a physiologically active preparation of vitamin B_{12} is disclosed that is free of pseudo or inactive vitamin B_{12}.

Similar teachings are also found in the documents GB 925 526 A and GB 1 007 972 A.

The document US 6 492 141 B1 pertains to the industrial production of vitamin B_{12} by using *Propionibacterium*.

The document US 2006/0051323 A1 discloses a food that has a particle that possesses a microbial biomass and a solid carrier, wherein the biomass comprises vitamin B_{12}. The biomass can include for example *Propionibacterium,* preferably *P. freudenreichii*. The biomass, produced in a spray-dried form, can be used as a food additive.

The document WO 2012/143 469 A1 discloses a food product in the form of a bar that comprises solid particles, for example consisting of cereal grains as well as vitamin B_{12} in the form of various cobalamins.

The document WO 2009/124 529 A2 discloses a method for producing a refreshing drink that is obtained with microbiologically formed cobalamin by additionally inoculating a cobalamin-synthesising strain of a microorganism. In this regard *Torulopsis glabrata, Lactobacillus lactis (lactis) and Kluyveromyces lactis* for example are particularly preferred as the cobalamin-synthesising strain of microorganism.

The document JP 5814629 2 A teaches the microbial production of vitamin B_{12} in high yield using *Propionibacterium freudenreichü* and *Propionibacterium shermanii.*

The unpublished patent application DE 10 2013 100891.7 discloses a method for producing an acid malt, a mash, a wort, a drink, a foodstuff, a soft water and a malt and the corresponding products. In this regard the products comprise vitamin B_{12} that at least to a high degree is bioavailable for the human and/or animal body.

The Offenlegungsschrift EP 2 548 948 A1 discloses a product, obtainable by lactic acid fermentation of a substrate by a strain of the species *Lactobacillus*

plantarum, in particular FERM ABP-11349 or FERM ABP-11350, which can achieve a cell count of 10^8 cfu/g in a pure orange juice or grapefruit juice.

The Offenlegungsschrift WO 2013/084 052 A1 discloses a product, obtainable by lactic acid fermentation of a substrate by a strain of the species *Lactobacillus reuteri* as the vitamin B_{12} producer, selected from the group consisting of DSM 23877, DSM 23878, DSM 23879 and DSM 23880.

The Offenlegungsschrift WO 2014/118 191 A1 discloses a method for producing a foodstuff with the following steps: preparing a mash or a wort or last runnings as a first nutrient medium; treating the first nutrient medium with lactic acid bacteria of the type *Lactobacillus coryniformis,* the type *Lactobacillus backii* (DSMZ Nr. 18080), the type *Lactobacillus plantarum* or the type *Lactobacillus fermentum* (DSMZ Nr. 20052) or with a mixture of at least two types of these lactic acid bacteria.

It would, however, be desirable if the amount or concentration of vitamin B_{12} comprised in the products produced with the last discussed method could be further increased.

Reference is hereby made to the unpublished patent application DE 10 2013 100891.7 in its entirety, such that its complete disclosure, in particular the technical features and effects cited therein, becomes part of the disclosure of this Application and of the invention described herein.

Object of the Invention

The object of the present invention is to provide an improved method for producing a foodstuff or a precursor of the same, in particular an acid malt, a mash, a wort, a drink or a non-fluid foodstuff, and the corresponding foodstuff or a precursor of the same, wherein this foodstuff or already a precursor of the same is suitable to improve the vitamin B12 supply of the human and/or animal body when this foodstuff or a precursor of it is ingested.

One aspect of the present invention is to provide a foodstuff or a precursor of the same which comprises an increased amount or concentration of vitamin B_{12}.

One aspect of the present invention is to provide a foodstuff or a precursor of the same which has been produced naturally and/or whose raw materials are permitted pursuant to the German Purity law or satisfy the German Purity law.

A further aspect of this invention is to fulfil the preceding object and/or at least one of the preceding defined aspects in a simple technical and cost-effective manner, in particular with the equipment that is commonly available in a brewery.

Summary of the invention

The object according to the invention is achieved by the subject matters of claims 1 to 13 as well as by the following defined subject matters.

In terms of the process technology the object according to the invention is achieved by a method for producing a foodstuff or a precursor of the same according to claim 1. The method has at least the following steps:

(a) Providing a first nutrient medium, preferably of a mash or a wort, in particular a first wort, or last runnings; and

(b) Treating the first nutrient medium with lactic acid bacteria of the species *Lactobacillus coryniformis,* in particular the sub-species *Lactobacillus coryniformis* subsp. *coryniformis* (DSMZ no. 20007), the species *Lactobacillus backii* (DSMZ Nr. 18080), the species *Lactobacillus plantarum* (preferably DSMZ no. 2601 or 2648 or 13273), in particular the sub-species *Lactobacillus plantarum* subsp. *plantarum* (DSMZ no. 20174) or *Lactobacillus plantarum* subsp. *argentoratensis* (DSMZ no. 16365), or the species *Lactobacillus fermentum* (DSMZ no. 20052) or with a mixture of at least two of these species of lactic acid bacteria.

The treatment according to step (b) occurs in the presence of a yeast product, wherein the yeast product comprises an extract, an autolysate and/or a dried form of a yeast or consists of an extract, an autolysate and/or a dried form of a yeast. According to the invention the yeast product may also be or comprise any mixture of an extract, an autolysate and/or a dried form of a yeast. The yeast product is preferably present in a concentration by weight in the range ≥ 0.2 and ≤ 20 g/l based on the first nutrient medium.

Treating the first nutrient medium with the previously cited lactic acid bacteria affords a medium, in particular an acid malt, which comprises vitamin B_{12}.

Surprisingly the amount and/or concentration by weight of vitamin B_{12} produced in the first nutrient medium can be increased by the presence of a yeast product or a combination of the described yeast products during the treatment of the first nutrient medium with lactic acid bacteria of the cited species.

In particular, as of an addition or concentration by weight of the yeast product of about 0.2 g/l (based on the first nutrient medium) an increase in the produced amount and/or concentration by weight of vitamin B_{12} can be observed, even when the yeast product as such comprises no vitamin B_{12}. In this regard the additionally produced amount of vitamin B_{12} increases about linearly with the concentration by weight of the yeast product present during the treatment, at least over a certain concentration range.

A heavy formation of sediment occurs in the reaction vessel above about a 20 g/l concentration by weight of the yeast product (based on the first nutrient medium), and can lead to losses in quality in the produced foodstuff (or in a precursor of the same) in regard to the odour and taste. This is presumably due to an overloading with cellular material and its decomposition products.

If the yeast product is a dried yeast then unpleasant odours may develop with high loadings, especially with loadings above 20 g/l. This is probably due to the increased release of fatty acids and/or their decomposition products. In addition, the technical implementation of the claimed method becomes increasingly uneconomic when high amounts of yeast products are added, in particular with concentrations by weight above 20 g/l.

According to the invention the first nutrient medium can be for example a nutrient broth, a mash, a wort, preferably a first wort, or first runnings.

Particularly with the use of mashes or worts as the first nutrient medium the applicant surprisingly determined that the inventively employed lactic acid bacteria generate more vitamin B_{12} in this type of medium and especially in the presence of a yeast product and are therefore suitable for a production of vitamin B_{12}. It was further determined that the vitamin B_{12}, produced by means of the

inventively employed lactic acid bacteria, is also bioavailable in the meaning of this Application.

When the method according to the invention is used then a further increased production of vitamin B_{12}, in particular bioavailable vitamin B_{12}, can therefore be achieved, apparently without "switching" the metabolism of the inventively provided lactic acid bacteria to produce unwanted substances. This type of switching is known for example for lactic acid bacteria of other species, such as for example *Lactobacillus reuterii.*

Advantageous embodiments of the method according to the invention are the subject matter of the dependent claims.

Thus, the first nutrient medium may be free or essentially free of hop bitter substances; and/or the yeast product may be free or essentially free of hop bitter substances.

The first nutrient medium and/or the yeast product may be taken from the brewing process. In particular, the first nutrient medium may be a cast wort or starting wort. Furthermore, the yeast product may be a pure selected yeast, for example for hopped wort, or a harvested yeast, for example from the first, second or third running. For these cases the inventors have discovered that the first nutrient medium and/or the yeast product may apparently comprise inhibitors that may be detrimental to the production of vitamin B_{12} by the proposed lactic acid bacteria species. This probably concerns the hop bitter substances.

According to the findings of the inventors the hop bitter substances can be completely or essentially completely removed from the yeast product, for example by washing the yeast product once or more with water, in particular with tap water or brewing water. The addition of such a yeast product that has been freed of hop bitter substances in the method according to the invention leads to an increase in the production of vitamin B_{12}.

Similarly, it could be determined that an at least partial inhibition of the production of vitamin B_{12} in the method according to the invention when the first nutrient medium was not free or not essentially free of hop bitter substances. Consequently, a mash or unhopped wort, for example a first wort or unhopped

cast wort or starting wort, which are free of hop bitter substances, is advantageously employed as the first nutrient medium.

Furthermore, the yeast product can be obtained from a top-fermenting or bottom-fermenting yeast of the genus *Saccharomyces*, in particular of the species *Saccharomyces cerevisiae* or of the species *Saccharomyces carlsbergensis*. In this regard the yeast is preferably a pure selected yeast or a harvested yeast from the beer production process. Further, the hop bitter substances are completely or essentially completely removed from the yeast product, preferably by washing the yeast or yeast product once or more with water, in particular with tap water or brewing water.

As the yeast product is obtained from a conventional brewery top-fermenting or bottom-fermenting yeast, especially from a harvested yeast, then in the case that the method according to the invention is carried out in a brewery a cost-effective and practically unlimited source is available for the raw material of the yeast product.

Moreover, experiments have demonstrated that a yeast product obtained by using the cited yeasts has a particularly positive effect on the production of vitamin B_{12} in the method according to the invention.

In addition, the method can further have the steps:

(e) providing a second nutrient medium, preferably of a mash or a wort, especially a cast wort; and

(f) mixing the medium obtained in step (b) with the second nutrient medium.

By mixing the medium obtained in step (b) with a second nutrient medium, preferably with a mash or wort, then a biologically acidified mash or wort can be produced by a simple, additional process step. The technological advantages of the biological mash or wort acidification are known to the person skilled in the art. Over and above the conventional advantages, the method according to the invention also affords a mash or wort with an increased content of bioavailable vitamin B_{12}.

When a mash or wort is used as the second nutrient medium the produced vitamin B_{12} advantageously remains essentially quantitatively and is not taken up again or metabolised by the lactic acid bacteria. Moreover, the vitamin B_{12} remains essentially completely or at least is bioavailable to a large extent. Thus, a mash or wort can be produced that in comparison to conventional mashes or worts possesses a considerably increased content of vitamin B_{12} without being over-acidified.

Thus, a mash produced according to the invention can exhibit a pH in the range of 4.5 to 5.7, preferably 4.9 to 5.3. A wort produced according to the invention can exhibit a pH in the range of 4.2 to 5.7, preferably 4.6 to 5.0.

The adjustment of the pH of the mash or wort to the cited values results in technological advantages, such as for example improved taste stability, foam stability and lighter colour of the beer.

The method can furthermore have the steps:

(j) preferably lautering the medium obtained in step (b) or (f),

(k) mashing out or keeping warm and preferably hopping the medium obtained in step (f) or (i);

(l) at least partly removing the lees from the medium obtained in step (k); and

(m) preferably adjusting the temperature of the medium obtained in step (l) to a pitching temperature.

The use of the steps (i) to (m) enables the biologically acidified mash or wort according to the invention to be further processed to a fermentable cast and pitching wort that likewise possesses an increased content of bioavailable vitamin B_{12}.

Thus, the method can also possess the step:

(p) processing the medium obtained in one of the steps (b), (f), (k), (l) or (m) to a drink, preferably treating this medium with a yeast of the genus

Saccharomyces, especially with the species *Saccharomyces cerevisiae* or the species *Saccharomyces carlsbergensis.*

The precursors obtained in the various process steps can be inventively further processed in a conventional manner, for example by alcoholic and non-alcoholic fermentation, to a drink that likewise has an increased content of bioavailable vitamin B_{12}. In particular, this drink can be: an alcohol-free drink, a beer, in particular an alcohol-free beer, a wheat beer, in particular an alcohol-free wheat beer, a beer-containing drink, in particular a mixed beer drink, a drink fermented or not fermented with a yeast. The drink according to the invention possesses an increased content of bioavailable vitamin B_{12}.

In spite of the inventively further increased content of vitamin B_{12} in the finished drink, the findings of the inventor show that an impairment or a decrease of the bioavailability does not or essentially does not occur in the additional process steps up to the finished drink. Accordingly, the same advantages of the inventively treated first nutrient medium, in particular of the produced acid malt, also analogously apply to the inventively produced drink, especially for a beer and an alcohol-free beer.

The method can furthermore have the steps:

(q) processing the medium obtained in one of the steps (b), (f), (k), (l) or (m) or the drink obtained in step (p) to a non-fluid, especially solid, food;

wherein the medium obtained in one of the steps (b), (f), (k), (l) or (m) or the drink obtained in step (p) is mixed with a precursor of the non-fluid food.

The precursors obtained in the various process steps or the drink can be inventively further processed in a conventional manner, for example by concentrating and/or mixing with other components, to a non-fluid foodstuff that likewise has an increased content of bioavailable vitamin B_{12}. In particular, this foodstuff can be: a cereal-containing foodstuff, in particular a cereal-containing bar, breakfast cereals, a malt extract product, a pastry, a dairy product, especially a yoghurt.

Consequently, the advantages of the inventively produced drink apply analogously to the abovementioned non-fluid foodstuff according to the invention.

The mass fraction of water in the medium that was obtained in one of the steps (b), (f), (k), (l) or (m), in the drink obtained in step (p) or in the non-fluid food obtained in step (q) can be adjusted to less than 35 %, preferably to less than 30 %, preferably to less than 25 %, preferably to less than 20 %, especially to less than 15 %.

If the water contents are higher than those indicated above then the microbiological stability cannot be guaranted for the foodstuff or a precursor thereof produced with the method according to the invention. If the water content is adjusted by removing water then the transport and storage costs will be reduced for the concentrated medium due to the reduction in volume.

Moreover, the concentrated medium can be rediluted to the original concentration or made up to a desired concentration anywhere and independently of the production site.

Furthermore, the viscosity of the concentrated medium is higher than that of the starting material, and is thereby advantageous when the concentrated medium is used to produce foodstuffs. For example, the concentrated medium can serve as a binder for granular or powdered components of foodstuffs.

Furthermore, it is advantageous if the mass fraction of the water in the inventively produced medium or drink is adjusted to more than 0 %, in particular to more than 5 %.

Dust formation in the course of processing or handling the foodstuff or its precursors is avoided by adjusting the residual water content to a predefined level.

The object according to the invention is also achieved by the subject matter of the product claim 9. In this claim a food or a precursor of the same is claimed, which is produced with a method according to one of claims 1 to 8.

In this regard the advantages cited in the description of the production method according to the invention apply analogously to a food or a precursor of the same produced by means of this method, in particular for an acid malt, a mash, a wort, a drink or a non-fluid food.

The object according to the invention is also achieved by the subject matter of the use claims 10 and 11.

The use of a yeast product is claimed in a method for the preparation of a food or a precursor of the same, preferably in a method according to one of claims 1 to 8. The method has at least the following steps:

(a) Providing a first nutrient medium, preferably of a mash or a wort, in particular a first wort, or last runnings; and

(b) Treating the first nutrient medium with lactic acid bacteria of the species *Lactobacillus coryniformis*, in particular the sub-species *Lactobacillus coryniformis* subsp. *coryniformis* (DSMZ no. 20007), the species *Lactobacillus backii* (DSMZ no. 18080), the species *Lactobacillus plantarum* (preferably DSMZ no. 2601 or 2648 or 13273), in particular the sub-species *Lactobacillus plantarum* subsp. *plantarum* (DSMZ no. 20174) or *Lactobacillus plantarum* subsp. *argentoratensis* (DSMZ no. 16365), or the species *Lactobacillus fermentum* (DSMZ no. 20052) or with a mixture of at least two of these species of lactic acid bacteria.

Wherein the treatment of step (b) takes place in the presence of the yeast product. Furthermore, the yeast product comprises an extract, an autolysate and/or a dried form of a yeast. The yeast product may also consist of an extract, an autolysate and/or a dried form of a yeast.

Also, the use is claimed for a method for preparing a food or a precursor of the same according to one of claims 1 to 8 so as to increase the bio-availability and/or the produced quantity and/or the concentration by weight of vitamin B_{12}, preferably of the bioavailable vitamin B_{12}, in the food or in the precursor of the same.

The advantages cited in the description of the production method according to the invention apply analogously to the uses according to the invention, including their specified embodiments. In addition, all the features disclosed in relation to the method according to the invention are also combinable with the use according to the invention.

In this regard the yeast product can be present in a concentration by weight in the range ≥ 0.2 and ≤ 20 g/l based on the first nutrient medium.

Also, the first nutrient medium can be free or essentially free of hop bitter substances. In addition or alternatively, the yeast product can be free or essentially free of hop bitter substances.

Alternatives and further disclosure of the invention

The invention is not limited to the use of *Lactobacillus coryniformis,* in particular *Lactobacillus coryniformis* subsp. *coryniformis*. The above described production method as well as the products may be carried out or produced alternatively with lactic acid bacteria of the species *Lactobacillus backii, Lactobacillus plantarum* or *Lactobacillus fermentum* or with a mixture of at least two of these species or the disclosed sub-species of lactic acid bacteria. The above described advantages and characteristics are thereby realised in an analogous manner.

According to the invention the mass concentration or the content of the yeast product based on the first nutrient medium may also be limited to the range of more than 0.2 to less than 10 g/l. Sediment formation in the reaction vessel is reliably avoided by choosing the upper limit of 10 g/l.

Also, the mass concentration or the content of the yeast product based on the first nutrient medium, in particular for the dried form of the yeast, may also be limited to the range of more than 5 to less than 10 g/l. In this concentration range the production of vitamin B_{12} increases strongly, wherein the above discussed disadvantages associated with increased yeast concentrations do not or only minimally occur.

If a yeast extract or a yeast autolysate is selected as the yeast product then its mass concentration may be chosen to be in the range of more than 3 to less than 15 g/l in order to obtain a high increase of the vitamin B_{12} production and simultaneously an acceptable quality of the resulting food or its precursors.

In this case, the mass concentration range may also be limited to more than 0.2 to less than 4 g/l. In this concentration range there is already a significant increased production of vitamin B_{12}, wherein the disadvantageous aspects

associated with the high mass concentration ranges are completely absent. Moreover, the vitamin B_{12} production in this range is particularly economic.

Having said that, a mass concentration range for a yeast extract or a yeast autolysate of more than 4 to less than 10 g/l may also be chosen. In this range the increase of the vitamin B_{12} production turns out to be particularly high, whereas the negative aspects associated with the high yeast product concentration occur only minimally – if at all. Thus in this range a high vitamin B_{12} yield can be achieved with an acceptable odour or taste quality of the resulting food or its precursors.

Advantageously, a precursor of the yeast product, in particular a top-fermented or bottom-fermented pure selected yeast or yeast harvested from the brewery, can be washed once or a plurality of times with water or with another suitable substance. This is carried out for example by suspending the yeast cells in the water and centrifuging. After the supernatant liquid has been thrown away the thus-washed yeast mass can be suspended again in water and then centrifuged again. The resuspension and centrifugation steps can be repeated at will until a desired purity has been achieved, in particular when the yeast is free or essentially free of hop bitter substances.

Advantageously, a first wort from the brewery is chosen as the first nutrient medium, wherein the first wort in step (a) has an extract content in the range 7 to 28 %, preferably 10 to 22 %, preferably 12 to 19 %, especially 14 to 16 %.

First wort as the first nutrient medium is characterised by a high content of the lactic acid bacteria used in the method according to the invention and required by the foods. Moreover, first wort is readily available and is easily produced with the equipment of a conventional brewery.

In addition, a wide concentration range in regard to the extract content of the first wort can be employed, thereby bringing flexibility to the application of the method.

The method according to the invention is also applicable to highly concentrated nutrient media that are obtained for example by high gravity methods, thereby achieving the thus-associated advantages, in particular volume and cost savings.

In an advantageous embodiment the first nutrient medium, prior to the treatment with the lactic acid bacteria, is diluted with water, especially brewery water, such that the extract content from the resulting dilution is in the range of 5 to 10 %, preferably 6 to 9 %, especially 7 to 8 %.

By diluting the first nutrient medium the optimal concentration of nutrients can be adjusted for the microorganisms employed in the method according to the invention.

In addition, the wastage of the first nutrient medium being produced can be reduced.

The first nutrient medium can be inoculated with the lactic acid bacteria in such an amount that the optical density (OD) of the first nutrient medium immediately after the inoculation is in the range of 0.1 to 1.0 OD, preferably 0.2 to 0.8, preferably 0.3 to 0.7, preferably 0.4 to 0.5 OD, especially about 0.45 OD, wherein the measured value of the optical density is measured at a wavelength of 620 nm and adjusted for the influence of the first nutrient medium.

The inoculation of the first nutrient medium with the inventively used microorganisms, such that the abovementioned optical density (OD) or a corresponding cell density is adjusted at the beginning of the treatment, leads to a rapid conversion and hence to an advantageously short treatment time.

Moreover, an optimal inoculation concentration of the lactic acid bacteria results in an optimal aroma profile with minimal concentrations or the complete absence of off-aromas.

An optical density of the first nutrient medium of less than 0.4, preferably 0.3, preferably 0.2 and especially 0.1 immediately after inoculation and optional homogenisation adversely results in too long a period until an envisaged, high conversion rate of the lactic acid bacteria is achieved.

In contrast, an optical density of the first nutrient medium of more than 0.5, preferably 0.7, preferably 0.8 and especially 1.0 immediately after inoculation and optional homogenisation causes sub-optimal growth conditions for the lactic acid bacteria, for example feedback inhibition.

The lactic acid bacteria at the beginning of the treatment of the first nutrient medium, especially when added to the first nutrient medium, according to step (b) can be found in the log phase or growth phase.

An inoculation with microorganisms that are in the log phase (logarithmic phase) or growth phase, due to the high activity of the added microorganisms, leads advantageously to a rapid conversion of the first nutrient to the acid malt.

The treatment according to step (b) can last between about 5 and 50 hours, preferably 20 to 48, especially 30 to 44 hours, especially 36 to 42 hours.

The inventively provided duration of treatment may be advantageously limited to the abovementioned short durations. In this way a short-term preparation of a treated first nutrient medium, especially of an acid malt, can be realised. In particular, with a treatment period of less than 5 hours the yield of vitamin B_{12} and/or lactate is too low. On the other hand, a treatment period of more than 50 hours results in no further increase in the production of bioavailable vitamin B_{12}. There is also the danger of over-acidification.

The treatment according to step (b) may be carried out at a temperature of the nutrient medium from about 15 to 48 °C, preferably 25 to 42 °C, in particular 32 to 40 °C, especially 35 to 39 °C, especially 36 to 38 °C.

The treatment according to step (b) may be advantageously carried out in a broad temperature range. The choice of a temperature between 30 and 40 °C creates optimal growth conditions for the microorganisms used, whereby the required treatment time is shortened and an optimal quality of the resulting products is maintained.

The method may also have a step, in which the first nutrient medium treated in step (b) is sterilised.

A subsequent sterilisation step can ensure that the inventively employed lactic acid bacteria cannot have an undesirable influence on the respective product when the product is subsequently used, for example in the context of the production of foods or drinks, in particular for the production of beer.

Thus, particularly for the use of yeast in a later process step, its metabolic activities will not be adversely affected.

The sterilisation may be carried out with all means known to the person skilled in the art. The addition of the acid malt to the boiling or hot wort is preferred.

The medium obtained in one of the steps (b), (f), (k), (l) or (m), the drink obtained in step (p) or the non-fluid food obtained in step (q) can have a mass fraction of lactic acid of about 0.1 to 1.0 %, preferably 0.2 to 0.6 %, preferably about 0.3 to o.5 %, especially about 0.35 to 0.45 %.

Due to the presence of lactic acid in the listed mass fractions the inventively produced medium, especially the acid malt, can be advantageously used to set a specific pH, for example of the mash or wort. This enables the pH of foods or of the corresponding precursors of the same, particularly of mash or wort, to be adjusted in a natural manner and in accordance with the Purity law.

When step (f) is carried out the second nutrient medium can have a temperature of at least 50 °C, preferably at least 60 °C, preferably at least 70 °C, preferably at least 80 °C, in particular at least 90 °C, especially at least 95 °C.

By adding the medium obtained in step (b) to the second nutrient medium at a high temperature there results an effective one-step inactivation or sterilisation of at least a part of the lactic acid bacteria. This obviates a specific sterilisation step together with the associated costs, time and energy.

The medium obtained in step (b) can be added in a volume fraction of 2 to 20 %, preferably 5 to 15 %, preferably 6 to 12 %, preferably 7 to 11 %, particularly 8 to 10 %, based on the volume of the resulting mixture.

For the mash or wort an optimal pH of 4.2 to 5.5, preferably 4.6 to 5.3, can be achieved in the resulting mixture, by adding such volume fractions of the acid malt.

The treatment of the first nutrient medium with lactic acid bacteria according to step (b) can take place under essentially anaerobic conditions, in particular under anaerobic conditions.

The choice of anaerobic conditions or essentially anaerobic conditions during the treatment of the nutrient medium with the inventively added lactic acid bacteria possibly has a positive effect on the quantity and/or bioavailability of the produced vitamin B_{12}.

The anaerobic conditions are preferably produced by excluding air and/or by degassing with CO_2 or N_2 or by other known measures.

The inventively produced foodstuff, particularly the drink, can be in conformity with the German Purity law or be produced exclusively from ingredients that are approved by the German Purity law for the production of beer. Accordingly, the raw materials for the foodstuff or drink according to the invention can be restricted to the raw materials approved by the German Purity law for brewing beer, in particular barley malt, wheat malt and brewery water, together with the inventively added microorganisms. Thus, for the first time it is inventively possible to prepare a foodstuff, in particular a drink, with an increased mass concentration of bioavailable vitamin B_{12}, which meets the Purity law.

The inventively produced foodstuff can have a gel-like or pasty consistency. A gel-like or pasty consistency advantageously favourises a more rapid or better resorption of the nutrient comprised therein, in particular the vitamin B_{12}, in the human or animal body. A gel-like or pasty consistency also provides an improved compatibility and an easy edibility or handling, for example for consumption during sport or leisure activities.

The inventively produced foodstuff can have a vitamin B_{12} mass fraction of at least 0.15 µg per portion, preferably at least 0.2 µg per portion, preferably at least 0.3 µg per portion, preferably at least 0.35 µg per portion, preferably at least 0.4 µg per portion, preferably at least 0.5 µg per portion, preferably at least 0.6 µg per portion, preferably at least 1.0 µg per portion, in particular at least 1.5 µg per portion of the foodstuff, wherein one portion of the foodstuff weighs 20 g.

The higher the vitamin B_{12} mass fraction in the foodstuff according to the invention the better is the achievable supply of vitamin B_{12} to the human or animal body.

The inventively produced foodstuff can have a mass ratio of glucose to fructose of 1.7:1 to 2.3:1, preferably 1.8:1 to 2.2:1, preferably 1.9:1 to 2.1: 1, in particular about 2:1.

This further improves the physiological action.

The inventively produced foodstuff can have a sodium ion mass fraction of at least 20 mg per portion, preferably at least 25 mg per portion, preferably at least 30 mg per portion, preferably at least 40 mg per portion, in particular at least 50 mg per portion of the foodstuff, wherein one portion of the foodstuff weighs 20 g.

The inventively produced foodstuff in concentrated form can have a sodium ion mass fraction of 50 to 200 mg per portion, preferably 60 to 150 mg per portion, in particular 70 to 100 mg per portion of the foodstuff, wherein one portion of the foodstuff weighs 20 g.

An adjustment of the inventively provided mass fraction of sodium ions further improves the physiological effect of the foodstuff, in particular the alleviation or mitigation of muscle cramps, in particular when the foodstuff according to the invention is employed as a sports nutritional product. A sodium ion mass fraction below the above cited figures does not provide sufficient physiological effect.

A sodium ion mass fraction of less than 200, preferably less than 55, in particular less than 50 mg per portion may potentially not have a sufficient physiological action. On the other hand, a sodium ion mass fraction of more than 100, preferably more than 120, in particular more than 135, potentially has a laxative effect on consumers.

β-Glucan or β-glucan-containing additives may be added to the foodstuff or to one of its precursors. The foodstuff can have a β-glucan mass fraction of at least 0.25 g per portion, preferably at least 0.3 g per portion, in particular at least 0.4 g per portion of the foodstuff, wherein one portion of the foodstuff weighs 20 g.

Due to its characteristic as a dietary fibre the addition of β-glucan to the foodstuff according to the invention improves the physiological effect of the foodstuff according to the invention, in particular in diets for active sporting lifestyles.

The inventively produced foodstuff may comprise cereal components, preferably malted and/or unmalted brewing grain, in particular barley malt and/or wheat malt.

Particularly with the use of mashes or worts as the first nutrient medium the applicant surprisingly determined that the inventively employed lactic acid bacteria generate more vitamin B_{12} in this type of medium and are therefore suitable for a production of vitamin B_{12}. It was further surprisingly determined that the vitamin B_{12}, produced by means of the inventively employed lactic acid bacteria, is also bioavailable to a high extent in the meaning of this Application. Also, the use of the above defined alternative method therefore results in an active production of bioavailable vitamin B_{12}, apparently without "converting" the metabolism of the inventively provided lactic acid bacteria to produce non-bioavailable vitamin B_{12}.

The cited features relating to the invention described in this Application, when not otherwise mentioned or evidently, illustrate optional features of advantageous embodiments that may be combined with any of the herein described subject matters and among one another, insofar as the person skilled in the art does not see any obvious drawback. Consequently, in particular, all features of the method presented in this description may also be combined with the products described in this Application, and vice versa. In particular, all features cited in relation to a product according to the invention are transferable and hence combinable with all other products described in this Application. This applies analogously to all methods and their features described in this Application. This applies analogously to the effects and advantages achieved by means of the described features.

Examples

1. Eliminating inhibitors, especially hop bitter substances, from the yeast by washing

A top-fermenting or bottom-fermenting pure selected yeast or a harvested yeast was suspended in brewing water in the ratio 1:9 (50 g yeast + 400 ml water). The resulting suspension was centrifuged at 1000 G for 5 minutes. The supernatant liquid was then discarded and the yeast sediment was re-

suspended in 150 ml brewing water. Both of the last steps can be repeated two or three times.

After washing, the yeast exhibited a clean, fresh, fruity odour. The original bitter note was absent. The yeast cells appeared to be intact in the microscopic preparation. Sporadic damaged cells were seen only in the bottom-fermenting yeast. The washed yeast cells exhibited a homogeneous plasma in addition to a large, round cell core. No breakup of the cell walls was observed. The cells were still colourless (= living) after vital staining.

2. Preparation of a yeast autolysate

The procedure for yeast autolysis is preferably initiated by bursting the cells. To do this the cells were treated mechanically, thermally or chemically. The autolysis then proceeded during an incubation of the yeast for a period of hours to days at 40 to 55 °C and at a suitable pH, preferably at a pH of 5 to 7.

A top-fermenting or bottom-fermenting pure selected yeast or a harvested yeast was propagated in cast wort. The dry substance fraction of the yeast was about 16 to 17%. It was freshly harvested and washed according to the above described method.

To burst the yeast cells they may optionally be pretreated by one or more of the following measures:

a) Wet cell disruption by means of a high pressure homogeniser;

b) Ultra-sound treatment (with and without glass beads);

c) Vortexing (with and without glass beads); and

d) Addition of propionic acid.

Details of the individual pretreatment methods are mentioned below.

The actual autolysis of the yeast cells was then carried out by incubating the optionally pretreated yeast cells at ca. 53 °C for 24 hours in the incubator (regulated amplitude: 50 to 55 °C) under continuous stirring of the batch. This afforded a liquid autolysate.

3. Preparation of a yeast extract

The liquid autolysate as produced above was concentrated down by removing water. If needed it was filtered and freed from adversely tasting substances.

The major constituents of the thus-obtained yeast extract are peptides and amino acids, resulting from the decomposition of the proteins, together with purine and pyrimidine, which result from the enzymatic cleavage of the nucleic acids.

4. Pretreatment details

a) Wet cell disruption by means of a high pressure homogeniser;

The mechanical disruption of the yeast cells was carried out with the high pressure homogeniser PANDA Plus 2000 from the GEA Niro Soavi company (Germany). Wet cell disruption by a high pressure homogeniser represents the preferred disruption method.

As a consequence of specific flow dynamics a local static negative pressure (500 to 1 500 bar, preferably 800 to 1 200 bar) is produced in the employed homogeniser. This leads to bubble formation both inside the yeast cell as well as on the interface between the yeast cell wall and the surrounding medium (cavitation effect). If the negative pressure is later spontaneously released at a relief valve this causes the bubbles to implode. This is followed by a punctual rupture of the cell walls.

The yeast samples were prepared by diluting the freshly cultivated brewery yeast with bewery water (1:2 v/v), decarbonated on the magnetic stirrer and then washed three times according to the above described method.

Each yeast sample passed twice through the described disruption process. The light microscopic inspection of the treated cells showed that after the cells had been subjected to the homogeniser they no longer contained protoplasts and only the cell envelopes remain.

The thus-disrupted yeast was then incubated in the incubator at about 53 °C for about 24 hours and autolysed by the still intact enzymes. To stop the autolysis the batches were boiled for about 30 minutes.

Alternatively the following processes may be employed for the cell disruption:

b) Ultra-sound (with and without glass beads)

The yeast sample is diluted with brewery water (10:90 v/v) and then subjected to ultra-sound for 10 minutes (ultra sound bath: MERCK eurolab USR 46 H).

Small glass beads (Prolabo/VWR Company, diameter of 2.5 to 3.5 mm) can be added to the yeast sample during the ultra-sound treatment in order to reinforce the mechanical forces.

(c) Vortexing by means of reagent glass shakers (with and without glass beads)

The yeast sample is diluted with brewery water (10:90 v/v) and then intensively rotated or stirred by means of a reagent glass shaker ("vortexing"; Vortex Genie 2-Schüttler: Bender & Hobein AG, Step 8).

Small glass beads (Prolabo/VWR Company, diameter of 2.5 to 3.5 mm) can be added to the yeast sample during the vortexing in order to reinforce the mechanical forces.

d) Addition of propionic acid

Propionic acid is added to the yeast sample, such that the acid content in the mixture is 5 vol. %.

The batch is manually rotated and then rotated by means of a vortexer. The batch is then shaken up and down for 15 minutes (TURBULA-Schüttler). The samples are left for 2 hours at room temperature and shaken again.

5. Preparation of dried yeast and yeast flakes

The yeast suspension (dry substance content of about 15 %) is uniformly sprayed onto a hot cylinder (cylinder dryer, VITAM GmbH, Hameln). The yeast cells burst on contacting the cylinder surface. The cell wall and cell contents dry out on the cylinder and after 3 seconds at the most are scraped off as flakes. About 1.5 kg flakes are obtained from a 10L yeast suspension. The yeast flakes are then comminuted in a morter.

6. Experiments with commercially available yeast products

For each batch 1 litre of an industrially manufactured, diluted first wort (50:50, v/v with brewery water), 15 ml of a bacterial suspension (*Lactobacillus coryniformis* subsp. *coryniformis* (DSMZ no. 20007), propagated in MRS broth (or, if specified: in a first wort), and 4 g of a yeast product (if hereinafter nothing else listed) was added into a 1 litre Schott flask and nomogenised (yeast product mass concentration: ca. 4 g/l). In the respective control was added the diluted first wort and the bacteria suspension, but no yeast product (insofar as nothing else was added). The batches were incubated for 24 hours at 37 °C in the incubator. All batches are based on a double determination and were repeated several times.

The quantities of the vitamin B_{12} formed in the above described batches (measured with the microbial method of r-Biopharm AOAC-Method no. 101002) are presented as the mass concentrazions in the following Table:

Batch	Commercial yeast product	Vitamin B_{12} mass concentration
		(µg/100 ml)
0	Control /without yeast product)	0.32
1	Beer yeast dry (tablets)	0.81
2	Beer yeast dry (flakes)	0.99
3	Molasses yeast dry (flakes)	1.28
4	Molasses yeast dry (flakes)	> 1.80
5	Molasses yeast dry (flakes)	> 1.80
6	Yeast extract granulated	2.56

All the commercial yeast products initiate a greater formation of vitamin B_{12} than does the control, although in different degrees. The production of vitamin B_{12} in the presence of yeast products was about twice to eight times more than by the control.

As a negative control the yeast products themselves were checked: None of the yeast products had a detectable quantity of vitamin B_{12}.

7. Experiments with commercially available yeast products (variable quantities)

In another series of experiments the relationship between the quantity of the added yeast product and the produced vitamin B_{12} was investigated. Here, the yeast extract of batch 6 (granulated yeast extract) from the previous Table was

used as the yeast extract. The other experimental design and the vitamin B_{12} determination method were identical with the above described experimental series.

Batch	Added yeast product	Vitamin B12 mass concentration	Additional vitamin B_{12} production against the control	
	(g/l)	(µg/100 ml)	(µg/100 ml)	(%)
0	0 (control)	0.37		
1	0.2	0.45	+ 0.08	+ 22 %
2	0.5	0.54	+ 0.17	+ 46 %
3	1.0	0.86	+ 0.49	+ 132 %
4	2.0	1.19	+ 0.82	+ 222 %
5	4.0	1.79	+ 1.42	+ 384 %

As can be seen in the above Table there is an increase in the vitamin B_{12} production over that of the control by more than 20 % even for an added amount of 0.2 g/l of the yeast product. The vitamin B_{12} production increases linearly with increasing added amounts, at least in the range examined here (coefficient of determination $R^2 = 0.9854$).

8. Experiments with yeast flakes from brewery yeast

A commercial top-fermenting and bottom-fermenting brewery yeast were investigated in another series of experiments. The freshly harvested yeasts were essentially freed from the hop bitter substances by the above described washing method. The resulting yeast suspension that had a dry substance content of about 15 % was then dried by cylinder drying (max. contact time of the yeast cells with the cylinder: 3 seconds). The dried yeast flakes were pulverised in a mortar prior to being employed as the yeast product. The other experimental design and the vitamin B_{12} determination method were identical with the above described experimental series.

Batch	Yeast product from brewery yeast (flakes; 4 g/l)	Vitamin B12 mass concentration	Additional vitamin B_{12} production against the control
		(µg/100 ml)	(%)
0	Control (without yeast product)	0.29	
1	Top-fermenting yeast	0.39	+ 34 %
2	Bottom-fermenting	0.54	+ 86 %

3	Mixture of top and bottom fermenting yeasts	0.51	+ 76 %

As can be seen in the above Table the use of a brewery commercial yeast also affords a considerable increase of the vitamin B_{12} production by the lactic acid bacteria. In particular, it appears that the presence of bottom-fermenting yeast has a positive effect on the vitamin B_{12} production by the lactic acid bacteria.

9. Experiments with yeast autolysate from brewery yeast

Yeast autolysates from each of a commercial top-fermenting and bottom-fermenting brewery yeast were investigated in another series of experiments according to the above described method. For this the freshly harvested yeast cells were autolysed at 37 °C for 24 hours in the incubator. In one part of the batch the described autolysis was carried out without pre-treatment. In another part of the batch, prior to the autolysis, the cell walls were broken up by means of the above described wet cell disruption by a high pressure homogeniser. The vitamin B_{12} determination method was identical to that of the above described series of experiments. The results are presented in the following Table:

Batch	Yeast autolysate from brewery yeast	Vitamin B12 mass concentration
		(µg/100 ml)
0	Control (without yeast autolysate)	0.152
1	Autolysate from top-fermenting yeast without	0.144
2	Autolysate from bottom-fermenting yeast	0.122
3	Autolysate from top-fermenting yeast with cell	0.160
4	Autolysate from bottom-fermenting yeast with	0.178

As may be seen from the above Table, the use of an autolysate originating from an untreated commercial top-fermenting or bottom-fermenting brewery yeast at best slightly increases the vitamin B_{12} mass concentration in those batches, in which the yeast cells were mechanically broken up prior to the autolysis. The inventor considers that this result may perhaps be because the autolysate obtained from a commercial brewery yeast comprises substances that prevent the previously observed increasing effect of yeast products on the vitamin B_{12} production. Consequently, it could be the case that certain inhibitors, probably hop bitter substances, are present in the autolysate and effectively inhibit B_{12} induction. These inhibitors probably originate from the hopped pitching wort, with

which the yeast comes into contact during the propagation (pure selected yeast) and/or during the fermentation.

A separate investigation of the autolysates showed that under the conditions of the batches the vitamin B12 mass concentrations of the autolysates themselves lay below the limit of determination (< 0.030 mg/100 ml). Therefore, the autolysates from brewery yeast do not themselves introduce any appreciable amounts of vitamin B_{12} into the batches.

Based on these findings, in a series of experiments a bottom-fermenting brewery yeast was washed according to the above described method in order to essentially remove the hop bitter substances from the yeast. As in the preceding batch the yeast cells were autolysed at 37 °C for 24 hours in an incubator. Other conditions, including the vitamin B_{12} determination method, were identical to those of the preceding series of experiments. The results are presented in the following Table:

Batch	Yeast autolysate from brewery yeast	Vitamin B12 mass concentration
		(µg/100 ml)
0	Control 1 (without lactic acid bacteria and without yeast autolysate)	0.116
1	Control 2 (with lactic acid bacteria and without yeast autolysate)	0.182
2	Autolysate from washed, bottom-fermenting yeast	0.368

The above Table shows that in this experimental design the treatment of the nutrient medium first wort with lactic acid bacteria of the sub-species *Lactobacillus coryniformis* subsp. *coryniformis* results in a vitamin B_{12} mass concentration that is about 57 % more than that of the control (without lactic acid bacteria; compare batches 0 and 1).

When the conversion of the same nutrient medium occurs under the same conditions, but inventively in the presence of an autolysate from washed, bottom-fermenting yeast, then the vitamin B_{12} mass concentration is about double that of the batch without yeast autolysate (+ 102 %; compare batches 1 and 2).

10. Determination of bioavailable vitamin B_{12}

In another series of experiments the mass concentration of the produced acid malt (precursor for foodstuff) was measured analogously to the previous point 6

by means of the detection method "ADVIA Centaur" (111659 Rev. N, 2008-09; VB12 2/12). The results are presented in the following Table:

Batch	Subject	Mass concentration of IF-bound (bioavailable) vitamin B_{12} (pg/ml)
0	Control 1 (water)	0.000
1	Control 2 /first wort)	0.084
2	Acid malt (first wort, converted with L. c., without yeast product)	0.146
3	Acid malt (first wort, converted with L. c., in the presence of yeast extract, granulated, 4 g/l)	0.499
L. c. = *Lactobacillus coryniformis* subsp. *coryniformis*		

The above Table shows that the treatment of the nutrient medium first wort with *Lactobacillus coryniformis* subsp. *coryniformis* already results in a high mass concentration of bioavailable vitamin B_{12} compared to the controls.

In the presence of a yeast product under otherwise identical test conditions the mass concentration of bioavailable vitamin B_{12} is further increased. In this experiment the batch with granulated yeast extract afforded 3.5 times the mass concentration of the comparable control batch without yeast product (both converted with lactic acid bacteria). Compared to the control without treatment with lactic acid bacteria (only first wort) almost 6 times the mass concentration of bioavailable vitamin B_{12} was achieved.

Comparison to the prior art

Conventionally, the species *Lactobacillus amylovorus* or *Lactobacillus amylolyticus* are used for the mash- or wort-acidification; these species have long proved successful for the production of corresponding acid malts and consequently acidified mashes and worts.

These lactic acid species are characterised by a growth dominance in beer wort due to rapid growth properties. Due to a high lactate production they also have a high acidification capacity. This is due to their homo-fermentative metabolic character. These species flourish at high temperatures (up to 52 °C), such that high propagation rates can be achieved.

Moreover, these species can ferment dextrin and starch. They also produce a high content of L(+)-lactate. It is of major importance that the cited lactic acid bacteria are not beer spoiling because they are sensitive to hops and cannot flourish at temperatures < 30 °C. Experts therefore consider *Lactobacillus amylovorus* and *Lactobacillus amylolyticus* to be suitable acidification organisms, because they do not form amines (histamine) or other toxins. Moreover, they do not form diacetyl or other substances that impair the taste and aroma of the resulting products. Finally, they are characterised by their ease of handling in practical use.

In contrast, the inventively provided species *Lactobacillus coryniformis* is considered by experts to be beer spoiling. This species flourishes in weakly hopped beer and produces diacetyl that leads to an adverse taste profile in the foodstuff or drink, particularly in beer. Moreover, *Lactobacillus coryniformis* is able to flourish at the typical fermentation temperatures for beer, particularly for top-fermented beer, namely in the temperature range of 15 to 48 °C. Furthermore, *Lactobacillus coryniformis,* in comparison to the conventional species *Lactobacillus amylovorus* and *Lactobacillus amylolyticus,* has the disadvantage of being facultatively hetero-fermentative, i.e. its acidification capability is reduced compared to the conventional species. Consequently, about twice the amount of acid malt must be employed compared to conventional species. This means that larger production equipment is required, and the costs associated with the acidification are greater.

The large number of disadvantages mentioned above, together with the disadvantages already known to the person skilled in the art in regard to the considered species *Lactobacillus coryniformis, Lactobacillus backii, Lactobacillus plantarum* and *Lactobacillus fermentum,* signified in the past an important obstacle to the use of these species or associated sub-species in the drink and foodstuff production industry, particularly in breweries and malt houses.

P A T E N T K R A V

1. Fremgangsmåde til fremstilling af et fødevaremiddel eller en forløber deraf, minimum med trinnene af:

(a) at tilvejebringe en mæske eller en urt eller et kavent, som et første fødevaremedium; og

(b) at behandle det første fødevaremedium med mælkesyrebakterier af arten Lactobacillus coryniformis, arten Lactobacillus backii (DSMZ Nr. 18080), arten Lactobacillus plantarum eller arten Lactobacillus fermentum (DSMZ Nr. 20052) eller med en blanding af mælkesyrebakterier af mindst to af arterne;

hvor behandlingen ifølge trin (b) sker i tilstedeværelse af et gærprodukt;

hvor gærproduktet indeholder et ekstrakt, en autolysat og/eller en tørret form af gær; og

hvor gærproduktet fortrinsvist foreligger i en massekoncentration i området af ≥ 0,2 og ≤ 20 g/l baseret på det første fødevaremedium.

2. Fremgangsmåde ifølge krav 1, k e n d e t e g n e t ved, at det første fødevaremedium er fri eller i det væsentlige fri af humlebitterstoffer; og/eller

gærproduktet er fri eller i det væsentlige fri af humlebitterstoffer; hvor "fri af humlebitterstoffer" med hensyn til det første fødevaremedium betyder et fuldstændigt fravær af humlebitterstoffer i det første fødevaremedium; hvor "fri af humlebitterstoffer" med hensyn til gærproduktet betyder et fuldstændigt fravær af humlebitterstoffer i gærproduktet betyder;

hvor "i det væsentlige fri af humlebitterstoffer" med hensyn til det første fødevaremedium betyder et indhold af humlebitterstoffer i det første fødevaremedium på højest 15 % baseret på indholdet af humlebitterstoffer, som en konventionel brygurt til gærpåsætning har til en fermentering med en undergær eller topgær med bitterenheder (EBC-fremgangsmåde) i området fra 15 til 38; og hvor "i det væsentlige fri af humlebitterstoffer" med hensyn til gærproduktet betyder et indhold af humlebitterstoffer i gærproduktet på højst 20 % baseret på indholdet af humlebitterstoffer, som et konventionelt undergær eller overgær til brygning har, hvilken høstes under en fermentering af en konventionel brygurt til gærpåsætning med bitterenheder (EBC-fremgangsmåde) i området fra 15 til 38.

3. Fremgangsmåde ifølge krav 1 eller 2, k e n d e t e g n e t ved, at gærproduktet er tilvejebragt fra overgær eller undergær fra slægten Saccharomyces, i særdeleshed arten Saccharomyces cerevisiae eller arten Saccharomyces carlsbergensis;

hvor gæren fortrinsvis er en rendyrket gær eller en høstet gær fra en ølproduktion;

hvor humlebitterstofferne fra gæret eller gærproduktet fuldstændig eller i alt væsentligt fjernes, fortrinsvis gennem vask af gæret eller gærproduktet med vand af en eller flere gange, i særdeleshed med drikkevand eller vand fra en brygning.

4. Fremgangsmåde ifølge et af kravene 1 til 3, k e n d e t e g n e t ved, at fremgangsmåden yderligere omfatter trinnene:

(e) tilvejebringe en mæske eller en urt, som et andet fødevaremedium; og

(f) blande mediet fra trin (b) med det andet fødevaremedium.

5. Fremgangsmåde ifølge et af kravene 1 til 4, k e n d e t e g n e t ved, at fremgangsmåden yderligere omfatter trinnene:

(i) fortrinsvis at klare mediet i trin (b) eller (f),

(k) at mæske eller at varmeholde og fortrinsvis at tilsætte humle i mediet i trin (f) eller (i);

(l) mindst at delvist fjerne bundfaldet fra mediet i trin (k); og

(m) fortrinsvis at regulere temperaturen af mediet i trin (l) til en gærpåsætningstemperatur.

6. Fremgangsmåde ifølge et af kravene 1 til 5, k e n d e t e g n e t ved, at fremgangsmåden yderligere omfatter trinnet:

(p) at oparbejde mediet fra et af trinnene (b), (f), (k), (l) eller (m) til en drikkevare, fortrinsvis at behandle mediet med en gær fra slægten Saccharomyces.

7. Fremgangsmåde ifølge et af kravene 1 til 6, k e n d e t e g n e t ved, at fremgangsmåden yderligere omfatter:

(q) at bearbejde mediet fra et af trinnene (b), (f), (k), (l) eller (m) eller drikkevaren fra trin (p) til et ikke-fluidt fødevaremiddel;

hvor mediet fra et af trinnene (b), (f), (k), (l) eller (m) eller drikkevaren fra trin (p) med sammenblandes med en forløber af det ikke-fluide fødevaremiddel.

8. Fremgangsmåde ifølge et af kravene 1 til 7, k e n d e t e g n e t ved, at fremgangsmåden yderligere omfatter trinnene:

(t) at indstille masseandelen af vand i mediet fra et af trinnene (b), (f), (k), (l) eller (m), i drikkevaren fra trinnet (p) eller det ikke-fluide fødevaremiddel fra trin (q) til mindre end 35 %, fortrinsvis mindre end 30 %, fortrinsvis mindre end 25 %, fortrinsvis til mindre end 20 %, i særdeleshed til mindre end 15 %; og

fortrinsvis til mere end 0 %, i særdeleshed mere end 5 %.

9. Fødevaremiddel eller en forløber deraf, fremstillet ved en fremgangsmåde ifølge et af kravene 1 til 8.

10. Anvendelse af et gærprodukt i en fremgangsmåde til fremstilling af et fødevaremiddel eller en forløber deraf, fortrinsvis i en fremgangsmåde ifølge et af kravene 1 til 8,

hvor fremgangsmåden mindst omfatter trinnene:

(a) at tilvejebringe en mæske eller en urt eller et kavent som et første fødevaremedium; og

(b) at behandle det første fødevaremedium med mælkesyrebakterier af arten Lactobacillus coryniformis, arten Lactobacillus backii (DSMZ Nr. 18080), arten Lactobacillus plantarum eller arten Lactobacillus fermentum (DSMZ Nr. 20052) eller med en blanding af

mælkesyrebakterier fra mindst to af arterne;

hvor behandlingen af trin (b) finder sted under tilstedeværelsen af gærproduktet; og

hvor gærproduktet indeholder et ekstrakt, en autolysat og/eller en tørret form af en gær.

11. Anvendelse af en fremgangsmåde til fremstilling af et fødevaremiddel eller en forløber deraf ifølge et af kravene 1 til 8 til at øge biotilgængeligheden og/eller den fremstillede mængde og/eller massekoncentrationen af vitamin B12 i fødevaremidlet eller forløberen deraf;

hvor "vitamin B12" er begrænset til en species, valgt blandt en gruppe, bestående af: methylcobalamin, desoxyadenosylcobalamin, hydroxycobalamin og sulfitcobalamin.

12. Anvendelse ifølge krav 10 eller 11, k e n d e t e g n e t ved, at gærproduktet foreligger i en massekoncentration i området fra ≥ 0,2 og ≤ 20 g/l baseret på det første fødevaremedium.

13. Anvendelse ifølge et af kravene 10 til 12, k e n d e t e g n e t ved, at det første fødevaremedium er fri eller i det væsentlige fri af humlebitterstoffer; og/eller

gærproduktet er fri eller i det væsentlige fri af humlebitterstoffer; hvor "fri af humlebitterstoffer" med hensyn til det første fødevaremedium betyder et fuldstændigt fravær af humlebitterstoffer i det første fødevaremedium; hvor "fri af humlebitterstoffer" med hensyn til gærproduktet betyder et fuldstændigt fravær af humlebitterstoffer i gærproduktet betyder;

hvor "i det væsentlige fri af humlebitterstoffer" med hensyn til det første fødevaremedium betyder et indhold af humlebitterstoffer i det første fødevaremedium på højest 15 % baseret på indholdet af humlebitterstoffer, som en konventionel brygurt til gærpåsætning har til en fermentering med en undergær eller topgær med bitterenheder (EBC-fremgangsmåde) i området fra 15 til 38; og hvor "i det væsentlige fri af humlebitterstoffer" med hensyn til gærproduktet betyder et indhold af humlebitterstoffer i gærproduktet på højst 20 % baseret på indholdet af humlebitterstoffer, som et konventionelt undergær eller overgær til brygning har, hvilken høstes under en fermentering af en konventionel brygurt til gærpåsætning med bitterenheder (EBC-fremgangsmåde) i området fra 15 til 38.