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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 July 2024 (11.07.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/147745 A1

(51) International Patent Classification:
C12C 12/00 (2006.01) *C12C 12/04* (2006.01)

(21) International Application Number:
PCT/PL2024/000001

(22) International Filing Date:
05 January 2024 (05.01.2024)

(25) Filing Language: Polish

(26) Publication Language: English

(30) Priority Data:
P.443422 05 January 2023 (05.01.2023) PL

(71) Applicant: **DRINK ID SP. Z.O.O.** [PL/PL]; ul. Browarna 5, 97-300 Piotrków Trybunalski (PL).

(72) Inventors: **SUPADY, Mariusz**; ul. Chabrowa 3, 97-300 Piotrków Trybunalski (PL). **SZYMANSKA, Monika**; ul. Piliczna 123, 97-213 Smardzewice (PL).

(74) Agent: **DOMON, Magdalena**; Kancelaria Prawnicza Piotr Korolko, ul. Żurawia 45, 00-680 Warszawa (PL).

(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).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2024/147745 A1

(54) Title: BEER AND BEER PRODUCTION METHOD

(57) Abstract: Beer simultaneously containing inactivated yeast and live probiotic bacteria cultures. A method of obtaining beer is characterized in that the base beer containing no more than 0.5% by volume of ethanol and a hop bitterness level no higher than 30 IBU for non-alcoholic variants, or a base beer containing up to 6% by volume of ethanol and a hop bitterness level no higher than 30 IBU for alcoholic variants: is subjected to flow pasteurization and then is cooled, dosed with carbon dioxide and live bacteria of the genus Lactobacillus and/or Bifodobacterium are dosed into the beer and then undergoes probiotic fermentation, during which live cells multiply.

Beer and beer production method

The subject of the invention is beer and the method of production of beer for use in particular as a health-promoting, functional beverage that improves or positively influences the quality of the microflora of the human digestive system.

Beer is the third most widely consumed beverage in the world, after tea and coffee, and is also the most preferred alcoholic beverage. The continued development of the brewing industry has helped the beverage gain a prestigious position.

At the same time, consumer expectations of the quality of both alcoholic and non-alcoholic beer regarding its composition, sensory properties and health-promoting potential continue to rise. The market is increasingly offering beers containing bioactive additives or minerals. Functional beers play a special role in the beer market.

For example, a non-alcoholic functional beverage based on a malt base or non-alcoholic beer or water-and-aromatic base is known from patent EP 3806656, which contains in its composition at the same time an autolysate obtained from waste yeast slurry and a fiber obtained from fragmented brewers' grains. The method of manufacturing such a functional beverage is also indicated.

As defined by the American Dietetic Association, a functional food can be defined as any product that has properties that have a positive effect on the human body, in addition to basic nutritional values. The purposeful impact of consuming functional foods as a source of physical and mental well-being is also emphasized. These foods contribute to preventing or reducing the risk of contracting particular diseases or improving the body's physical functions.

Moderate beer consumption has been shown to reduce the risk of cardiovascular disease by 20 ÷ 40%, including the incidence of coronary artery disease, heart attacks, as well as strokes. It has also been observed to reduce the risk of ulcers, gallstones and kidney stones or arthritis. The effect of beer consumption can also be calming, reducing stress or making it easier to fall asleep. Composition of the beer, as well as its beneficial effects on the human body, indicate that it is a good base for developing valuable functional products.

Hence, breweries are introducing bioactive additives, minerals, or removing gluten from beer. [[BARBARA STACHOWIAK, KRZYSZTOF BUKOWSKI, ŻYWNOŚĆ. Nauka. Technologia. Jakość, 2021, 28, 1 (126), 5 – 27].

New alcohol-free functional beers and alcoholic beers with more favorable properties compared to beers currently known on the market are being sought, in particular beers that contain live probiotic bacteria.

Probiotic bacteria are a group of microorganisms that naturally inhabit the digestive tract (intestines) of a healthy person; they include strains of the genus Lactobacillus and Bifidobacterium.

It has been shown that probiotic bacteria can have a very positive effect on the human body because:

- they facilitate the digestive process,
- they increase the absorption of vitamins and minerals,
- with antibiotic treatments, they protect our intestinal microflora,
- they affect the immune system by increasing resistance to infection,
- some strains have anti-allergic and anti-cancer effects,
- they lower cholesterol levels,
- they alleviate the symptoms of lactose intolerance,
- they have the ability to synthesize some B vitamins, vit. K, folic acid.

Many beverages containing probiotic bacteria are known in the state of the art. The best known are drinks such as sours, including bread and beetroot-based, home brew, and a whole range of dairy products containing lactic acid bacteria in their composition, such as kefir, sour milk, buttermilk, yogurt, acidophilus milk. Probiotic bacteria are also contained in sauerkraut, pickled cucumbers, and pickled beets.

Due to the positive health-promoting properties of probiotic bacteria, products beyond probiotic dairy products, or pickles, are being sought.

However, due to the peculiarities of beer production, especially the technological requirements, it is a huge challenge to produce beer containing probiotic bacteria.

The state of the art describes several ways to produce sour beer fermented with microorganisms belonging to lactic bacteria (or with lactic bacteria added after the alcoholic fermentation process conducted with yeast). However, they have the disadvantage of being made in such a way that they can only produce sour beer with the characteristic taste and smell of pickles. At the same time, it is not possible to produce non-alcoholic beer or bottom- or top-fermented beer by a similar method.

Similarly, previous attempts to use probiotic bacteria in beers have resulted in only one type, i.e. sour beer which, due to its sour taste, differs significantly from traditional, popular beers. For example, from patent application WO 2018/182512, a sour beer containing probiotic bacteria is known, which is produced in the following steps: supplying wort, adding probiotic bacteria to the wort, adding yeast to the wort, fermenting the wort for a set time and at a set temperature until an alcoholic beverage is formed. This patent provides a process for making a fermented product, where the use of different probiotic bacterial strains and different yeast strains, not always yeasts considered brewing varieties (of the genus Saccharomyces), is revealed in various examples. It is also advisable to use yeasts isolated from the environment, considered wild, such as Pichia or Mietschnikowia. At the same time, in each case indicated, it is a process leading to the formation of an alcoholic product, especially a sour one, and there is no way to obtain non-alcoholic beer by the disclosed method.

There are also sour craft beers made according to old recipes. Unlike in a modern brewery, where brewing is done under sterile conditions to protect the beer from the influence of wild yeasts, these sour beers allow wild strains of yeast or bacteria to participate. Craft beers are alcoholic beers that contain live lactic fermentation microorganisms and also contain live yeast, herbs and spices.

However, what all these products have in common is fermentation occurring in succession or simultaneously, using yeast and probiotic bacteria or lactic bacteria (typically acidifying). Each of the products listed is an alcoholic product. In addition, the solutions known from the state of the art do not allow to brew bottom- or top-fermented beer, in which probiotic bacteria would be multiplied.

Also, a disadvantage of the solutions known from the state of the art is the presence of live yeast in the product. For products that contain live yeast cells in their composition, there may be an increase in alcohol and carbon dioxide during the shelf life due to the metabolic activity of the yeast. In addition, too much carbon dioxide can cause risks such as distortion of the can, or plastic packaging. In addition, such a solution containing live yeast cells makes it impossible to accurately determine the alcohol content of the final product, hence while it could be applicable to craft breweries, the lack of stability/repeatability of the composition during shelf life would be difficult to accept for larger-scale production.

Bacteria that are considered probiotic are acidifying bacteria and in the standard beer production process they represent yeast infection. Hence, the process of obtaining probiotic beer requires maintaining very high hygienic standards, so as not to infect standard process yeast with probiotic microflora. This production aspect is a significant difficulty in making probiotic beer, and thus technological processes are being sought to produce stable probiotic beer.

Another difficulty in obtaining probiotic beer is the sensitivity of probiotic bacteria to some of the components typical of beer such as high ethanol content and the content of bitter substances in hop.

Hence, non-alcoholic and alcoholic beers with the flavor typical of bottom- or top-fermented beers without the aftertaste characteristic of sourdough, while containing live probiotic bacteria, are also in demand.

Bottom-fermented beers are beers fermented with bottom-fermenting yeast, which are characterized in that after the fermentation process is completed, they settle to the bottom

of the fermentation tank, from where they are collected, and this is the opposite behavior to top-fermenting yeast, which remains after the process on the surface of the fermented liquid; beers prepared with their use are called top-fermented beers. This is the main difference between the two processes. Both top-fermented beers and bottom-fermented beers are made using pure culture noble yeast, and it is a monoculture, meaning one type of yeast is used in a given process, which is propagated from a slope of pure culture. Sour beers are beers in the production of which different types of microorganisms, i.e. yeast and bacteria, are used in a single fermentation process. The fermentation process generally begins with a yeast fermentation stage followed by a bacterial fermentation stage. As a result of yeast-bacterial metabolic activity, beers prepared in this way have a much higher acidity and lower pH level than classic top- and bottom-fermented beers. Sour beers are often beers of so-called spontaneous fermentation, where the fermentation process involves yeast and bacteria inoculated from the air, rather than pure cultures propagated from slopes of pure cultures.

The process of fermenting beer, regardless of the type of fermentation, is preceded by the stage of creating beer wort, which is the liquid that undergoes the fermentation process.

The purpose of the solution according to the invention is to provide a non-alcoholic or alcoholic beer containing live probiotic bacteria cultures, and at the same time a beer that will have the same properties during its entire shelf life, and the same alcoholic content in the alcoholic version. The invention also aims to provide functional beer.
In addition, the aim of the invention is to provide a beer that will have the taste of a classic bottom/upper fermentation beer, which, despite the content of live probiotic microorganisms, will not have the aftertaste characteristic of sourdough.

The purpose of the invention is also to prepare the beer base in a suitable manner and to provide a product containing within it live cultures of the genus Lactobacillus and/or Bifidobacterium.

According to the invention, beer based on a bottom-fermented beer base or based on a top-fermented beer base is characterized in that it simultaneously contains inactivated yeast and live probiotic bacteria cultures in its composition.

A beer base is a beer to be inoculated with probiotic microorganisms. The beer base is the basic ingredient of the beer prepared according to the invention. The beer base can be a type of bottom- or top-fermented beer.

Bottom- or top-fermented beers known in the state of the art contain: malt extract in an amount ranging from 6%-15% m/v of finished product (in which malt extract barley malt extract may range from 60-100% m/v and wheat malt extract may range from 0-40% m/v), hop extract in an amount ranging from 0.01% - 0.3% m/v, and ethanol in an amount ranging from 0.01- 6% v/v. The beers may contain inactivated yeast in an amount of less than 0.1% m/v, preferably less than 0.01% m/v, while the beers may also contain aromas characteristic of a given type of beer, all topped off with water to 100%.

Malt extract are substances that are carried out from the solid fraction (grain, malt) to the water fraction at the wort mashing stage at the brewhouse.

Hop extract are bittering and aromatic substances that go from the solid fraction to the liquid during the boiling of wort with hop. Hop extract contains isomerized hop acids (bittering and aromatic substances).

Advantageously according to the invention, the level of hop bitterness in beer is no higher than 30 IBU.

The bitterness content of hop is determined in International Bitterness Units (IBUs, for short) established by the European Brewery Convention. IBUs indicate the degree of bitterness in a beer. 1 IBU corresponds to 1 milligram of iso-alpha acids derived from hop in 1 liter of beer.

In the solution according to the invention, the level of bitterness can range from 0 to 30 IBU. For a beer that does not contain hop bitterness (0 IBU), then the beer is usually flavored with herbs other than hop, while for a beer that contains hop up to 30 IBU, then it is hopped.

Advantageously according to the invention, the base beer (beer base) is produced by a bottom or top fermentation process.

Advantageously, the beer according to the invention is characterized in that it contains up to 0.5% alcohol.

Advantageously, the beer according to the invention is characterized in that it contains more than 0.5% alcohol up to 6% alcohol.

Advantageously, the beer according to the invention is characterized in that it contains live cultures of probiotic bacteria of the genus Lactobacillus and/or Bifodobacterium.

According to the invention, the method of obtaining beer is characterized by a base beer containing no more than 0.5% by volume of ethanol and a hop bitterness level of no more than 30 IBU for non-alcoholic variants, and up to 6% by volume of ethanol and a hop bitterness level of no more than 30 IBU for an alcoholic product

- is subjected to pasteurization in the flow and then cooled to a temperature of 10°C to 20°C and directed to another intermediate tank.
  Pasteurization is aimed at eliminating yeast in the first place; then,
- it is dosed with carbon dioxide, preferably to a level of 4.5 g/l - 5 g/l
- live bacteria of the genus Lactobacillus and/or Bifodobacterium are dosed into the beer so as to obtain a minimum of 10^5 and preferably 10^6 live bacterial cells in 1 ml of beer. The contents of the tank are mixed using carbon dioxide so that the contents are evenly mixed.
- the product is directed to probiotic fermentation, during which live bacteria multiply to a level of 10^7 to 10^8 live cells per milliliter of beer, with the fermentation process taking place at temperatures ranging from 5°C to 30°C and lasting from 10 to 30 days.
  The fermentation process is monitored microbiologically through plate cultures in which the level of bacterial multiplication is controlled.
  The course of the fermentation process is monitored physically and chemically by controlling parameters such as pH, acidity, saturation, turbidity.

Advantageously, before the pasteurization stage, the beer is filtered; advantageously, the filtration is carried out with a candle filter.

Advantageously, the method according to the invention is characterized in that, in the case of the non-alcoholic version, the base beer is enriched with B vitamins such as vitamin B12 and/or vitamin B6 and/or niacin and/or pantothenic acid so as to achieve a level of 7.5% to 30% of the RDI (Reference Daily Intake) in 100 ml of beer. The RDI values are shown in Table 1.

The vitamin content helps the bacteria survive, responds to their requirements and improves their living conditions. The process can take place in an intermediary tank.

Advantageously, the method according to the invention is characterized in that, prior to the probiotic fermentation stage, the beer is directed to be bottled into individual packages, advantageously of the crown capped bottle or lid capped can type.

Advantageously, the method according to the invention is characterized in that, after the probiotic fermentation stage, the beer is directed to be bottled into individual packages, advantageously of the crown capped bottle or lid capped can type.

Advantageously, the method according to the invention is characterized in that the fermentation process takes place in the temperature range from 5°C to 18°C for a period of 10 to 30 days.

Advantageously, the method according to the invention is characterized in that the fermentation process takes place in the temperature range from 18°C to 30°C for a period of 5 to 10 days.

Advantageously, the method according to the invention is characterized in that the base beer is produced by a bottom or top fermentation process.

Table 1. Reference Daily Intake (RDI) values of B vitamins

Vitamin	RDI
Thiamine (mg)	1.1
Riboflavin (mg)	1.4
Niacin (mg)	16
Vitamin B6 (mg)	1.4
Folic acid (µg)	200

Vitamin B12 (μg)	2.5
Biotin (μg)	50
Pantothenic acid (mg)	6

During the fermentation process according to this invention (it is a probiotic fermentation process, i.e., such a metabolic process that takes place without oxygen or with very little oxygen present), changes in physical, chemical and sensory parameters occur due to the metabolic activity of live probiotic microorganisms. Turbidity and sediment on the bottom of the package appear and changes occur in the taste of the beer due to the appearance of bacterial metabolites such as pyruvic acid, lactic acid, malic acid, butyric acid, acetic acid and propionic acid. The product reaches fermentation maturity when microbial proliferation reaches a level of 10^7-10^8, and at a reduction in pH relative to the pH of the product at inoculation of 0.2 to 0.35.

During the fermentation process, for example, the pH may decrease by 0.25 to 0.35 from a starting level of about 4.8. Acidity, on the other hand, will increase slightly to 2.5-2.8 from a starting value of less than 2. Turbidity increases to values in the range of 20-80 EBC. The solution according to the invention has many advantages, including that in the ready-to-eat product the consumer does not receive live yeast, but only live probiotic bacteria. Unlike products that contain live yeast cells in their composition, in the product according to the invention there is no increase in the amount of alcohol and thus no increase in the amount of carbon dioxide during the shelf life due to the metabolic activity of the yeast.
In addition, the solution according to the invention provides assurance to the consumer that throughout the shelf life he will be able to consume a product with the alcohol content declared on the label (alcoholic version) or without alcohol.
It is also important that the invention provides the consumer with a classic beer, in a non-alcoholic or alcoholic version, rather than a sour beer or a product with an undefined taste resulting from fermentation with wild yeast.

Hence, the solution according to the invention provides alcoholic beer or non-alcoholic functional beer containing live cultures of probiotic bacteria, with the resulting product being a final unpasteurized product containing live probiotic bacteria cultures. Both the alcoholic and non-alcoholic versions of the solution according to the invention are sources of live

probiotic microorganisms.

Advantageously, the beer according to the invention contains:

- malt extract in the amount of 6%-15% m/v
- ethanol in the amount of 0.01% to 6% v/v
- hop extract (bittering and aromatic substances) in the amount of 0.01%-0.3% m/v
- live probiotic microorganisms in an amount of not less than $1*10^7$ per 0.5 liters,
- additives and formulation-specific flavors in an amount of 0 to 1.5% m/v, preferably 0.5% to 1% m/v,
- inactivated yeast at less than 0.1% m/v,
- composition make-up water to 100% m/v.

Advantageously, the beer according to the invention contains inactivated yeast in an amount of 0.01% to 0.1% m/v.

Advantageously, the beer according to the invention contains inactivated yeast in an amount of less than 0.01% m/v.

In addition, the resulting product has a taste typical of bottom/upper fermentation beers, and through the technological procedure used, live probiotic microorganisms are maintained in the product without the aftertaste characteristic of sourdough.

A consumer consuming non-alcoholic or alcoholic beer with live cultures of probiotic bacteria, in addition to excellent taste and thirst quenching, provides the body with live probiotic microorganisms, in an amount of no less than 10^6 per 1 ml of the product, which support the microflora of the human digestive tract positively influencing the overall functioning of all vital processes of the human body.

The method of making beer according to the invention is shown in non-limiting performance examples.

The basis for preparing an alcoholic or non-alcoholic beer with live probiotic bacteria cultures, or base beer, can be any type of alcoholic or non-alcoholic beer. In the case of non-alcoholic beer in particular, these may be beers obtained by non-alcoholic fermentation of the malt base or by dealcoholization or any other known method of producing non-alcoholic beer.

In non-limiting performance examples, the base beers that are the basis for the preparation of probiotic beer are presented.

EXAMPLE 1 (Base beer for preparation of probiotic beer)

Non-alcoholic malt beer obtained by fermentation using dedicated yeast, for example, Saccharomycodes ludwigii pure culture, containing no more than 0.5% by volume of ethanol after the fermentation process. In order to obtain 0.5% ethanol by volume, the mashing process of the malt base was modified to reduce the amount of fermentable sugars produced, that is, the conditions for amylolytic enzyme activity were eliminated by conducting the mashing at conditions above 70°C. In addition, the fermentation process was modified to reduce the amount of ethanol formed by using a fermentation temperature of up to 16°C and a dedicated breed of yeast, such as Saccharomycodes ludwigii. It is a bottom-fermented beer. Wheat malt, barley malt and hop were used to produce the base beer at the brewhouse stage.

EXAMPLE 2 (Base beer for preparation of probiotic beer)

Non-alcoholic malt beer obtained by dealcoholization of standard alcoholic beer. Dealcoholization is carried out on distillation-column instruments that allow the evaporation of ethanol from alcoholic beer. As a result of the dealcoholization process, non-alcoholic beer is obtained, containing no more than 0.5% ethanol by volume.

Dealcoholization can be carried out on top- or bottom-fermented beer.

EXAMPLE 3 (Base beer for preparation of probiotic beer)

Light or dark beer, obtained by classical fermentation using Saccharomyces yeast, with an alcoholic strength of up to 6% by volume.

The beer can be top or bottom fermented; The beer in its composition contains for light beer: light barley malt and hop, and for dark beer contains light barley malt and caramel and roasted barley malts and hop.

Examples of the implementation of the invention:

Example 1: Non-alcoholic citrus-flavored probiotic lager beer with an extract content of 9.5 degrees Plato, an alcohol strength of up to 0.5% by volume and a live probiotic microbial content of no less than 10^6 live cells per milliliter was produced in the following stages:

- the base, non-alcoholic beer made according to the technological process described in EXAMPLE 1 with an extract of 9.5 degrees Plato was filtered through a diatomaceous earth filter,

- the filtered clear beer was then directed to flow pasteurization, during which it was subjected to a heat treatment of 72°C for 60 seconds, during which the yeast was deactivated, and after pasteurization was cooled on a heat exchanger to 15°C.

- vitamin B12 was then dosed into the beer in an intermediate tank at a rate of 0.5 μg per 100 ml of beer,

- lemon-lime flavoring was dosed to the volume of beer in the tank at a rate of 5 kg for every 10000 l of beer,

- a preparation of probiotic bacteria from the genus Lactobacillus containing 10^11 live microorganisms per gram of preparation was dosed into the volume of beer in the tank. The dosage amount was such as to obtain 10^6 live microorganisms in 1 ml of beer in the tank.

- the contents of the tank were thoroughly mixed with carbon dioxide. Samples were taken from the tank for physical-chemical and microbiological analysis; the carbon dioxide saturation of the tank was set at 4.5 g/l of beer, and the contents of the tank were directed to be bottled in crown capped glass bottle type containers. At the time of bottling, the product was not subjected to the pasteurization process in order not to damage the living microorganisms.

- after bottling, the product was allowed to ferment in a room at 15°C for 15 days.

- after this time, a sample of the product was taken for physical, chemical and microbiological analysis. The product at this stage was made available for sale because it had achieved microbial multiplication to a level of 10^8 and a reduction in pH by a value of 0.3 compared to the pH value in the beer on the day of bottling; the pH value on the day of bottling was 4.8. Turbidity increased from 1.3 EBC to 30 EBC, and acidity from 1.5 to 2.1. Samples of the product made available on the day of sale were set aside in the production archive to control the amount of live microorganisms during the shelf life of the product.

In practice, the shelf life of the product was no less than 6 months.

If microbial levels fell below the inoculation threshold, it would mean the end of shelf life.

Plato degrees are a measure of extract content by weight relative to 100 kg of base wort.

Example 2. Non-alcoholic dark probiotic beer with an extract content of 10° Plato, an alcoholic strength of up to 0.5% by volume and a live probiotic microbial content of not less than 10^6 live cells per milliliter was produced in the following stages:

- the base, non-alcoholic beer made according to the technological process described in EXAMPLE 2 with an extract level of 10 degrees Plato was filtered through a diatomaceous earth filter before the dealcoholization process,

- the dealcoholized beer was directed to flow pasteurization, during which it was subjected to a heat treatment of 80°C for 60 seconds, and cooled on a heat exchanger to 10°C after pasteurization,

- in an intermediate tank, vitamin B6 was dosed into the beer at 0.3 mg per 100 ml of beer and pantothenic acid at 2 mg per 100 ml of beer,

- a preparation of probiotic bacteria from the genus Bifidobacterium containing 10^11 live microorganisms per gram of preparation was dosed into the volume of beer in the tank.

The dosage amount was such as to obtain 10^5 live microorganisms in 1 ml of beer in the tank.

- the contents of the tank were thoroughly mixed with carbon dioxide. Samples were taken from the tank for physical-chemical and microbiological analysis; the CO_2 saturation of the tank was set at 4.7 g/l of beer, and the compactness of the tank was directed for bottling into can-type packages closed with a lid. At the time of bottling, the product was not subjected to the pasteurization process in order not to damage the living microorganisms.

- after bottling, the product was allowed to ferment in a room at 25°C for 10 days.

- after this time, a sample of the product was taken for physical, chemical and microbiological analysis. The product at this stage was made available for sale, as it achieved microbial multiplication to 10^7 and a pH reduction of 0.25 from that value in the beer on the day of bottling, with a pH of 4.85 on the day of bottling. Turbidity increased from 1.3 EBC to 80 EBC, and acidity from 1.5 to 2.5. Samples of the product released on the day of sale were set aside in the production archive to control the amount of live microorganisms during the shelf life of the product.

If microbial levels fell below the 10^6 threshold, it would mean the end of shelf life. In practice, the shelf life of the product was no less than 6 months.

Example 3. Alcoholic dark probiotic beer with an extract content of 8.5° Plato, an alcoholic strength of up to 3.5% by volume and a live probiotic microbial content of not less than 10^6 live cells per milliliter was produced in the following stages:

- the base, alcoholic beer made according to the process described in EXAMPLE 3 with an extract of 11.5 degrees Plato was filtered through a diatomaceous earth filter and directed to a clean intermediate tank.

- the beer was directed to flow pasteurization, during which it was subjected to a heat treatment of 85°C for 60 seconds, and cooled on a heat exchanger to 10°C after pasteurization,

- a preparation of probiotic bacteria from the genus Bifidobacterium and Lactobacillus containing 10^11 live microorganisms per gram of preparation was dosed into the volume of beer in the tank. The dosage amount was such as to obtain 10^7 live microorganisms from 1 ml of beer in the tank.

- the contents of the tank were thoroughly mixed with carbon dioxide. Samples were taken from the tank for physical-chemical and microbiological analysis; saturation of the tank was set at 4.7 g/l of beer, and the compactness of the tank was directed for bottling into can-type packages closed with a lid. At the time of bottling, the product was not subjected to the pasteurization process in order not to damage the living microorganisms.

- after bottling, the product was allowed to ferment in a room at 25°C for 10 days.

- after this time, a sample of the product was taken for physical, chemical and microbiological analysis. The product at this stage was made available for sale because it had achieved microbial multiplication to a level of 10^8 and a pH reduction of 0.25 from that in the beer on the day of bottling, with a pH value of 4.8 on the day of bottling. Turbidity increased from 1.3 EBC to 80 EBC, and acidity from 1.5 to 2.5. Samples of the product made available on the day of sale were set aside in the production archive to control the amount of live microorganisms during the shelf life of the product.

If microbial levels fell below the 10^6 threshold, it would mean the end of shelf life. In practice, the shelf life of the product was no less than 6 months.

PATENT CLAIMS

1. Beer based on a bottom-fermented beer base or based on a top-fermented beer base **characterized in that** it simultaneously contains inactivated yeast and live probiotic bacteria cultures in its composition.
2. Beer according to claim 1 **characterized in that** it contains hop bitterness of up to 30 IBU.
3. Beer according to claims 1-2 **characterized in that** the beer base (base beer) is produced by bottom or top fermentation.
4. Beer according to claims 1-3 **characterized in that** it contains up to 0.5% alcohol.
5. Beer according to claims 1-3 **characterized in that** it contains more than 0.5% alcohol up to 6% alcohol.
6. Beer according to claims 1-5 **characterized in that** it contains live cultures of probiotic bacteria of the genus Lactobacillus and/or Bifodobacterium.
7. Beer according to claims 1-6 characterized in that it contains:
   - malt extract in the amount of 6%-15% m/v
   - ethanol in the amount of 0.01% to 6% v/v
   - hop extract (bittering and aromatic substances) in the amount of 0.01% to 0.3% m/v
   - live probiotic microorganisms in an amount of not less than $1*10^7$ per 0.5 liters,
   - additives and formulation-specific flavors in an amount of 0 to 1.5% m/v, preferably 0.5% to 1% m/v,
   - inactivated yeast in an amount of less than 0.1% m/v, preferably less than 0.01% m/v
   - composition make-up water to 100% m/v.
8. A method of obtaining beer **characterized in that** the base beer containing no more than 0.5% by volume of ethanol and a hop bitterness level no higher than 30 IBU for non-alcoholic variants, or a base beer containing more than 0.5% to 6% by volume of ethanol and a hop bitterness level no higher than 30 IBU for alcoholic variants:
   - is subjected to flow pasteurization and then cooled to a temperature between 10°C and 20°C,
   - is dosed with carbon dioxide to a level between 4.5 and 5 g/l,

- live bacteria of the genus Lactobacillus and/or Bifodobacterium are dosed into the beer so as to obtain a minimum of 10^5 and preferably 10^6 live bacterial cells in 1 ml of beer, and then
- undergoes probiotic fermentation, during which live cells multiply to a level of 10^7 to 10^8 live cells per milliliter of beer, with the fermentation process taking place at temperatures from 5°C to 30°C and lasting from 10 to 30 days.

9. The method according to claim 8 is characterized in that before the pasteurization step the beer is filtered, advantageously the filtration is carried out by means of a candle filter.
10. The method according to claims 8-9, **characterized in that** it for the non-alcoholic version, the base beer is enriched with B vitamins, such as vitamin B12 and/or vitamin B6 and/or niacin and/or pantothenic acid, to achieve a level of 7.5% to 30% of the Reference Daily Intake in 100 ml of beer.
11. The method according to claims 8-10 **characterized in that,** prior to the probiotic fermentation stage, the beer is directed for bottling into individual packages, preferably of the crown capped bottle or lid capped can type.
12. The method according to claims 8-11 **characterized in that,** after the probiotic fermentation stage, the beer is directed for bottling into individual packages, preferably of the crown capped bottle or lid capped can type.
13. The method according to claims 8-12 **characterized in that** the fermentation process takes place in the temperature range from 5°C to 18°C for a period of 10 to 30 days.
14. The method according to claims 8-13 **characterized in that** the fermentation process takes place in the temperature range from 18°C to 30°C for a period of 5 to 10 days.
15. The method according to claims 8-14 **characterized in that** the base beer is produced by bottom or top fermentation.

INTERNATIONAL SEARCH REPORT

International application No
PCT/PL2024/000001

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12C12/00 C12C12/04
ADD.

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)
C12C

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	US 11 160 295 B2 (ERDINGER WEISSBRAEU WERNER BROMBACH GMBH & CO KG [DE] ET AL.) 2 November 2021 (2021-11-02) column 18 - column 20	1-15
A	TOH MINGZHAN ET AL: "Influence of commercial inactivated yeast derivatives on the survival of probiotic bacterium Lactobacillus rhamnosus HN001 in an acidic environment", AMB EXPRESS, [Online] vol. 7, no. 156, 24 July 2017 (2017-07-24) , pages 1-12, XP0055842379, DOI: 10.1186/s13568-017-0456-4 Retrieved from the Internet: URL:https://amb-express.springeropen.com/track/pdf/10.1186/s13568-017-0456-4.pdf> [retrieved on 2024-05-15] the whole document	1-15

☐ Further documents are listed in the continuation of Box C.

☒ 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
15 May 2024	03/06/2024

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	Merel-Rausch, Eva

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/PL2024/000001

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 11160295 B2	02-11-2021	DE 102017120283 A1	07-03-2019
		EP 3450534 A1	06-03-2019
		JP 6674514 B2	01-04-2020
		JP 2019058162 A	18-04-2019
		US 2019069579 A1	07-03-2019