

200255 MY

**Description**

**Microbiologically stabilised beer**

The present invention relates to agents and processes for the production of microbiologically stabilised beer.

In the last ten years or thereabout, a large number of small breweries, the so-called pub breweries, have been set up again which differ from large breweries above all by their small production volume which usually covers only the quantities being served in their own pub and direct sales of small containers such as bottles, and by a simpler brewing and bottling/racking process.

Whereas the large breweries in particular operate large industrial plants in which the production and bottling/racking of the beer is possible under germ inhibiting or low germ conditions, many of the small breweries lack corresponding precautions and measures. Structural or process technology measures leading to such advantageous conditions under which contamination with micro-organisms harmful to beer can be completely avoided are usually economically unattractive for small breweries.

On the other hand, it is especially the low germ production and bottling/racking which has a positive effect on the result of brewing; many of the micro-organisms entrained in production are "harmful to beer". A beer produced to be low in organisms harmful to beer is suitable for storage for longer periods and can even in an environment not completely germ free easily be bottled or racked in kegs without a negative effect having to be expected.

It is desirable to provide as simple and cost effective processes and means as possible which allow both breweries run on an industrial scale and small breweries to carry out production and bottling/racking of beer which has been microbiologically stabilised.

A risk of contamination with germs, i.e. of microbiological destabilisation, of the beer arises above all in connection with bottling of the beer, racking in kegs or filling into similar vessels. "Low germ" should not be understood to mean complete freedom from germs. The number of germs harmful to beer, in particular micro-organisms that are present in beer, such as bacteria and fungi, should be so low that the beer does not spoil during a prolonged storage period of weeks and months. If the number of germs harmful to beer is kept low, the probability that cultures harmful to beer develop is reduced. Preferably, bottling/racking takes place in a beverage-sterile or beer-sterile manner.

Processes for low germ bottling/racking of beer and other beverages are known from the state of the art. Low germ bottling/racking of beer can take place with an increased effort of biological monitoring. In this case, intensive cleaning and sterilisation measures are carried out on the bottling/racking facilities and the corresponding premises. These measures are accompanied by a large effort in terms of biological controls and involve a large amount of labour and high costs. Both for continual monitoring of low germ conditions and for maintaining them, complex and/or time-consuming measures in terms of equipment or process technology need to be taken in some cases. However, the assurance thus obtained is relatively small compared with the necessary effort/expenditure. Consequently, such measures are seldom employed. The process is not reliable enough for bottling/racking sugar-containing beer beverages such as mixed beer beverages or maltbeer which exhibit a particularly high risk of contamination.

A further measure for low germ bottling/racking of beer is high temperature short time processing (HTST). In this case, beer is heated by means of a high-temperature short-time pasteuriser, usually a plate heat exchanger by the continuous flow method and subsequently cooled again. Ideally, the HTST-facility is installed directly in front of the filling machine. Nevertheless, there is a risk of secondary contamination, for example during the bottling/racking process or by filling into containers which have already been contaminated with germs. HTST processing is a frequently used measure; however, it is not safe enough for bottling/racking of sugar-containing beer beverages.

A further measure for low germ bottling/racking of beer is membrane filtration or cold disinfection. Basically, the process corresponds to the HTST process, however, a membrane filtration system is used for germ reduction instead of a high-temperature short-term pasteuriser, which system allows micro-organisms present in the beer to be separated off as a result of the pore size of the filter. In this case, too, there is a risk of secondary contamination. The process is again not sufficiently reliable for bottling/racking of sugar-containing beer beverages.

A further measure is full pasteurising of bottles or cans which have already been filled and closed, preferably in a tunnel pasteuriser or chamber pasteuriser. In this process, the containers into which the beer has been filled are heated e.g. by means of hot water or steam and subsequently cooled again. The process is frequently used in breweries with long distribution channels, e.g. when exporting beer to countries overseas. The danger of secondary contamination during bottling/racking is thus controllable. In this way, sugar-containing beverages such as Malzbier or mixed beer beverages can be stabilised biologically with sufficient reliability. This process is cost intensive as a result of the large amount of equipment required. Apart from the high operating costs such as increased water and energy consumption, the high investment costs for plant and the high space requirement are a disadvantage. Moreover, the containers and closures (in particular the temperature and pressure stability) need to satisfy high requirements regularly. Plastic bottles made of PET currently commonly used in the beverage sector cannot be pasteurised according to known methods. A further disadvantage is the impairment of the taste caused by the pasteurisation process.

A further possibility for low germ bottling/racking of beer or for providing bottled/racked microbiologically stabilised beer are processes known from the juice and lemonade industry. These include chemical sterilisation. In the case of some lemonades, it is possible according to the law on food products to use the preservation dimethyl dicarbonate (DMDC) shortly before bottling. As a rule, this requires prior HTST treatment. When correctly used, the substance added continues to take effect in the filled and closed bottle and degrades after a certain period. A disadvantage is the very difficult technical application of the substance in the brewing plant since it is harmful to health and has a high freezing point. For sugar-containing beers such as mixed beer beverages or

maltbeer, the use of this substance is unsuitable since the permitted concentration can be used only in the concentrated lemonade portion or the lemonade base. In connection with beer bottling/racking, however, the substance would have to be added too early so that there is a risk that it is no longer fully effective in the filled bottle. The above-mentioned chemical sterilisation is consequently generally not suitable for beer bottling.

A further type of bottling/racking known from the field of the juice and lemonade industry is aseptic, i.e. sterile bottling/racking. This is associated with a considerable expenditure on equipment and plant facilities such as clean room and isolation technology. In addition, this process presupposes a changeover of the quality assurance concept including validation and employee qualification. The use for industrial scale beer bottling/racking as well as for bottling/racking from pub breweries is unrealistic because of the high technical and organisational effort/expenditure.

The technical problem on which the present invention is based consists essentially of providing processes and means for the production and/or bottling/racking of beer or mixed beer beverages which can be carried out easily and cost effectively and which more effectively reduce or stabilise the number of micro-organisms harmful to beer during and/or after production. In this way, a beer or mixed beer beverage microbiologically stabilised in the bottled/racked state is to be obtained. The processes and means must further be suitable for use in known beer production processes and brewing facilities without essential adaptation of the process steps. The technical problem is solved by providing a process for the production of beer or mixed beer beverage from brewing liquor, hops and at least one source of carbohydrate, wherein in a first step (a) brewing liquor, hops and the source of carbohydrate are mixed to form wort. In a subsequent step (b) the wort is boiled. In a subsequent step (c) the wort is microbially fermented. The process according to the invention is characterised in that the source of carbohydrate contains isomaltulose or an isomaltulose-containing mixture as germ-stabilising agent.

The invention thus teaches the production of beer or mixed beer beverages from brewing liquor, hops and a source of carbohydrate, wherein isomaltulose or an isomaltulose-containing mixture is contained in the source of carbohydrate

as germ-stabilising agent or the source of carbohydrate preferably consists of it. The inventors have surprisingly found that isomaltulose or an isomaltulose-containing mixture in the source of carbohydrate reduces the germ count of micro-organisms recognised as disadvantageous, i.e. harmful to beer, and/or microbially stabilises the beer obtained. By using isomaltulose or an isomaltulose-containing mixture, the number of these micro-organisms does not increase in the further production process and during bottling/racking such that, subsequently, a beer low in germs harmful to beer or a beer essentially free from germs harmful to beer is obtained which is microbially stabilised. Beer obtained and bottled/racked in this way is then suitable in particular for prolonged storage.

"Microbially stabilised" or "germ stabilised" should be understood here to mean that a food which is basically subject to contamination and decay possesses certain properties as a result of which further or undesirable growth of micro-organisms is suppressed or completely prevented. Such micro-organisms which are also referred to as germs consist above all of bacteria and fungi such as moulds or yeasts. These include not only those organisms which detrimentally influence quality and taste of the food as a result of their metabolic activity but also potentially pathogenic germs which may endanger the health of human beings. The germ stabilisation according to the invention leads to the number of such micro-organisms not rising above a certain threshold in appropriate storage periods such that the food is not spoilt or does not pose a risk to health.

In connection with the present invention, "beer" should be understood to mean – as the expert can easily recognise – not only a beer which is obtained after complete or almost complete fermentation of the wort, i.e. of the carbohydrate components contained therein. Beer should be understood to mean here also a mixed beer beverage which is obtained when at least one further carbohydrate component which is not or only partially fermented, is added to the beer before, during or after its production. For example, beer should be understood here to mean a mixed beer beverage in the case of which isomaltulose is added to conventionally produced beer during or after production.

Preferably, the germ-stabilising agent is added to a source of carbohydrate containing malted grain (malt), raw grain or a mixture of malted grain and raw

grain. In the source of carbohydrate used according to the invention, malt and/or raw grain is thus partially replaced by isomaltulose or by an isomaltulose-containing mixture. In a particularly preferred embodiment, the ratio of the other components of the source of carbohydrate, i.e. in particular malt and/or the raw grain, to the isomaltulose added is 6:1 to 1:1, preferably 4:1 to 2:1, particularly preferably, depending on the field of application, precisely 6:1, 5:1, 4:1, 3:1, 2:1 or 1:1.

So as to modify the known and proven processes for the production of beer as little as possible, isomaltulose or the isomaltulose-containing mixture is added to the source of carbohydrate, preferably before mixing with brewing liquor and hops, preferably as a syrup, as a solution and/or as a crystalline solid. In a further preferred variant, isomaltulose or the isomaltulose-containing mixture is added to the wort together with the other source of carbohydrate, the brewing liquor and hops.

Preferably, isomaltulose is added to the beer in the brewhouse and subsequently passes through the entire main and secondary fermentation process.

Alternatively, isomaltulose is added exclusively or additionally after main fermentation. The addition of isomaltulose after main fermentation ensures that the isomaltulose cannot be metabolised during main fermentation although, as a rule, a residual activity of the yeast is still present during secondary fermentation.

In a further preferred embodiment, isomaltulose is added to the beer additionally or exclusively only after filtration. If the isomaltulose is added to the beer after filtration, the mixture is not subjected to any modifications by the desired fermentation process as a result of the yeast being separated off.

In a further preferred embodiment, the isomaltulose is added additionally or exclusively directly before bottling/racking or before storage of the beer or mixed beer beverage.

In all the modalities of isomaltulose addition mentioned above, isomaltulose acts according to the invention as a germ-stabilising, germ-growth inhibiting or antigerminative agent. In any case, the isomaltulose addition, be it as a syrup, as a solution or as a crystalline substance, can be accommodated without difficulties into known beer production processes such that any additional effort in terms of process technology or equipment is avoided.

Particularly in the case of mixed beer beverages, which, as a result of the lemonade content, are more easily perishable as a rule, the use of isomaltulose in the beer part contributes to a considerable improvement of the biological stability of the mixed beer beverage. In addition, further known advantages such as suitability for diabetics of the mixed beer beverage produced are obtained.

Surprisingly, the known detrimental effect of taste deterioration which is known from other sugar substitutes or sweetening agents, does not occur in the case of the use, preferred according to the invention, of isomaltulose in mixed beer beverages. In particular, the addition of isomaltulose does not or only insignificantly change taste aspects such as palatibility in beer or mixed beer beverages.

A further subject matter of the present invention is consequently also a beer or mixed beer beverage in which isomaltulose is contained as sweetening agent. Preferably, isomaltulose is contained as the only sweetening agent. Further preferably, isomaltulose is contained as the only body-providing sweetening agent.

A subject matter according to the invention is consequently also the use of isomaltulose as germ-stabilising or germ-inhibiting agent for low germ production, above all bottling/racking of beers or mixed beer beverages, in particular according to the process according to the invention described here and its preferred modifications according to the invention.

Isomaltulose is a reducing sugar which surprisingly can be assimilated and metabolised by beer yeasts such as *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis* either not at all or only with great difficulty. Isomaltulose (6-O- $\alpha$ -D-glucopyranosyl fructose), known by the name of

Palatinose™, is a disaccharide ketose which occurs naturally e.g. in honey. According to DE 44 14 185 C1, isomaltulose can be produced from sucrose on an industrial scale by enzymatic rearrangement, using for example immobilised bacteria cells, in particular of the species *Protaminobacter rubrum*, *Erwinia rhapontici* and *Serratia plymuthica* or a sucrose isomerase isolated therefrom.

An "isomaltulose-containing mixture" is a combination of isomaltulose with at least one further carbohydrate, in particular fructose, glucose, sucrose, trehalulose, leucrose, tagatose, turanose, isomaltose, isomelizitose, oligosaccharides with a degree of polymerisation of 3 or 4 or more or mixtures thereof. In a modification, the mixture contains isomaltulose and fructose, in a further modification, the mixture contains isomaltulose and glucose, in a further modification the mixture contains isomaltulose and sucrose, in a further modification the mixture contains isomaltulose and trehalulose, in a further modification the mixture contains isomaltulose and leucrose, in a further modification the mixture contains isomaltulose and tagatose, in a further modification the mixture contains isomaltulose and turanose, in a further modification the mixture contains isomaltulose and isomaltose, in a further modification the mixture contains isomaltulose and isomelizitose, in a further modification the mixture contains isomaltulose and oligosaccharides with a degree of polymerisation of 3 or 4 or more. In a preferred embodiment, the isomaltulose-containing mixture is the sucrose-isomerisation product which has been obtained by transglucosidation of sucrose, preferably using dead or living cells of *Protaminobacter rubrum* or enzyme extracts produced therefrom. In a particularly preferred embodiment of the invention, isomaltulose-containing mixtures contain approximately 79 - 85 % isomaltulose, 8 - 10 % trehalulose, 0.5 - 2 % sucrose, 1 - 1.5 % isomaltose, oligosaccharide, 2.5 - 3.5 % fructose and 2.0 - 2.5 % glucose or they consist thereof, these details relating to the solids content as a percentage.

"Wort" should be understood to mean the extract liberated from insoluble components which consists of a carbohydrate source, e.g. malt, to which water and preferably hops have been added and which has been boiled. After boiling with hops, the so-called finished wort is obtained. After cooling, the boiled wort is available as pitching wort. Preferably, the wort is produced by mashing, lautering, wort boiling and wort treatment. The production of the wort has the

particular aim of converting initially undissolved components of the source of carbohydrate, in particular malt, into soluble fermentable substances, separating off the remaining solid components and finally adding seasoning, i.e. the hop. During mashing, the milled source of carbohydrate, in particular malt, is preferably first mixed with the brewing liquor. Subsequently, preferably in the so-called mashing processes, a targeted enzymatic conversion of constituents of the source of carbohydrate takes place in a specific temperature-time programme, the most important process being the complete decomposition of starch to fermentable sugars such as glucose, maltose or maltotriose and non-fermentable dextrans. The temperature optimum of maltose formation is 60 °C - 65 °C, that of dextrin formation 70 °C - 75 °C. The temperature determines the final fermentation of the wort depending on the type of beer. Following lautering and sweetening out of the spent grains with hot brewing liquor (78 °C), the wort is preferably boiled for 60 min to 100 min, preferably with the addition of hops, preferably approximately 150 to 500 g/hl of hops being added, depending on the type of beer to be produced. By evaporating preferably approximately 6 - 10 % of the feed quantity, the content of original wort is adjusted. During boiling, sterilisation additionally occurs, a coagulation of proteins takes place, hop bitter compounds are isomerised and aroma compounds are formed and partially evaporated. The boiled and hopped wort is subsequently preferably liberated from sediment particles in the whirlpool and/or by filtration. After cooling of the wort which usually takes place in plate heat exchangers, the cold break is preferably partially removed and an intensive aeration takes place to supply the micro-organisms used for fermentation with oxygen. Immediately subsequently, at least one suitable, fermentation-intensive micro-organism, for example yeast, is added to the wort. Since the wort used for fermentation may contain different sources of carbohydrate, pale or dark microbiologically stabilised beers can be produced by using the process according to the invention.

According to the invention, part of the extract of the wort is preferably replaced by isomaltulose. Thus, the proportion of metabolisable carbohydrates in the wort is reduced such that in addition the alcohol content of the beverage produced is preferably reduced compared with that of a normal beer. The alcohol content of the beers produced according to the invention can, if necessary, be further reduced by using alcohol removal processes. An "alcohol-

free beer" should be understood to mean a beer with an alcohol content of less than 0.5 % which preferably contains approximately 7 to 8 % of original wort (if not indicated otherwise, % values are to be understood as % by vol.). A "low-alcohol beer" should be understood to mean, according to the invention, a beer which has an alcohol content of less than 5 %, in particular less than 4 %.

A "source of carbohydrate" should be understood to mean materials containing carbohydrates such as grain products in the case of which the carbohydrates can be converted at least partially during the production of the wort into fermentable soluble sugars such as glucose, maltose or maltotriose which are then utilised as source of carbohydrates by micro-organisms, in particular yeasts, during fermentation. In a preferred embodiment of the invention, the source of carbohydrate used is malted grain, raw grain or a mixture thereof.

Malted grain preferably consists of grains and seeds of barley, wheat, rye, oats, millet, triticale, rice, sorghum and/or sweet corn which have been subjected to a malt production process. Raw grain preferably consists of grains and seeds of barley, wheat, rye, oats, millet, sorghum, triticale, rice and/or sweet corn, which have been milled, but not malted.

Preferably, the starting materials are saccharified before fermentation. For this purpose, the malt-inherent, hydrolytically active enzymes such as amylases, maltases etc., which convert starch into non-fermentable dextrins and fermentable glucose, maltose and maltotriose, are used. During malt preparation, the steeped cereals are allowed to germinate preferably at 12 °C to 18 °C and the germination process is interrupted as soon as the enzyme formation and dissolution processes have reached the desired degree. This takes place preferably by using elevated temperatures with a high throughput of air. By predrying at preferably 40 to 50 °C (withering), the water content can be reduced from more than 50 % to 10 to 12 %. Subsequently, the temperature can preferably be raised to approximately 80 to 85 °C in order to adjust the water content of the malt to preferably approximately 4 to 5 %. This process is referred to as kilning.

The fermentation process takes place preferably in two stages. The main fermentation is initiated by adding micro-organisms, in particular yeasts, bottom-

fermenting yeasts or top-fermenting yeasts. At the end of the main fermentation process, the yeasts separate off at the bottom or in the hopper of the fermentation vessel. The green beer obtained during main fermentation is preferably cooled down and subjected to secondary fermentation whereby the residual extract is fermented and the beer preferably clarified. During fermentation, the taste of wort also disappears as a result of which the pure beer taste is formed in particular during secondary fermentation. This process is also referred to as maturation. Fermentation can be influenced e.g. by different fermentation temperatures, top-fermenting and bottom-fermenting methods of production, open fermentation or closed fermentation etc.

Preferably, a single or several of the micro-organisms selected from a bottom-fermenting *Saccharomyces cerevisiae* strain, a top-fermenting *Saccharomyces cerevisiae* strain, *Saccharomyces carlsbergensis*, *Saccharomyces diastaticus* and *Schizosaccharomyces pombe*, is/are used for fermentation.

Preferably, microbiologically stabilised top-fermented or bottom-fermented beer is produced using the process according to the invention. Bottom-fermented beer is obtained in the case of bottom fermentation, wherein the yeast settles on the bottom of the vessel after fermentation and can be separated off from there. Top-fermented beer is a beer which is obtained by top fermentation, wherein the yeast rises upwards at the end of fermentation and is separated off at the top as far as possible.

In a further preferred embodiment of the invention, it is provided for the fermentation process to be carried out using at least one yeast and at least one acidogen selected from the group consisting of representatives of *Lactobacillus* sp., *Acetobacter* sp. and *Gluconobacter* sp. In a preferred aspect of this embodiment it is, for example, provided for the fermentation to be carried out using *S. cerevisiae* and/or *S. diastaticus* and/or *Schizosaccharomyces pombe* and a representative of *Lactobacillus*. Lactobacilli which are also known as lactic acid bacteria are capable of fermenting lactic acid. Low-alcohol or alcohol-free Beers or beer-like beverages produced as a result of such fermentation are characterised by a mild acidic taste which corresponds approximately to that of Berliner Weiße beer.

In a further preferred aspect of this embodiment, it is provided, for example, for the fermentation to be carried out using *S. cerevisiae* and/or *S. diastaticus* and/or *Schizosaccharomyces pombe* and a representative of *Acetobacter*. The species of *Acetobacter* comprises, in the narrower sense of the word, the acetic acid bacteria which are capable of forming acetic acid by the oxidation of ethanol. The low-alcohol or alcohol-free beers or beer-like beverages thus produced are given an acidic taste which is markedly different from the taste of the beverages obtained using *Lactobacillus*.

In a further preferred aspect of this embodiment, it is provided, for example, for the fermentation to be carried out using *S. cerevisiae* and/or *S. diastaticus* and/or *Schizosaccharomyces pombe* and representative of *Gluconobacter*. *Gluconobacter* is capable, on the one hand, of oxidising ethanol to acetic acid and, on the other hand, glucose to gluconic acid. The low-alcohol or alcohol-free beers or beer-like beverages produced by this mixed fermentation also have a pleasant acidic taste.

Consequently, subject matter of the present invention is also a microbiologically stabilised beer which can be produced according to the process described above and which is preferably produced according to this process.

A subject matter is also a microbiologically stabilised low-alcohol or alcohol-free beer, dietetic beer, malted drink, "Malzbier" or alcohol-free beer-like soft drink produced according to the invention. In a preferred embodiment, it is a pale microbiologically stabilised low-alcohol or alcohol-free beer or a dark microbiologically stabilised low-alcohol or alcohol free beer.

"Malted drink" should be understood to be a slightly hopped, carbon dioxide-containing and dark beverage with a predominantly malt-aromatic taste of malt sweetness which, moreover, is low in alcohol to free from alcohol. Preferably, the malted drink is brewed with approximately 7 - 8 % original wort from the malt content. After filtering, it is adjusted preferably with sweetening sugars (glucose, sucrose) to 12 % of original wort (approximately one third of the original wort).

Because of the advantages of isomaltulose in comparison with conventional sugar, i.e. sucrose, such as little sweetening strength, higher microbiological stability, suitability for diabetics, anticariogenic properties, an even higher proportion of isomaltulose can be chosen in the original wort.

A further subject matter is also a biologically stabilised mixed beer beverage which contains the microbiologically stabilised beer according to the invention and at least one further component selected from: extracts of herbs, aroma compounds, caffeine, colorants, amino acids, culinary acids, fruit components such as fruit juice, fruit flesh, fruit pulp or fruit extracts, sugar, sugar substitutes such as sugar alcohols, intensive sweeteners, water, distilled spirits (ethanol). Preferably, the mixed beer beverage consists of the microbiologically stabilised beer according to the invention and the at least one further component.

"Herb components" should be understood to mean: extracts, solutions, essences from parts of plants, preferably anise, valerian root, stinging nettle, blackberry leaves, strawberry leaves, fennel, lady's mantle, goose grass, ginseng, rosehip, hibiscus flowers, raspberry leaves, elderberry, hops, ginger, St.-John's wort, camomile, coriander, curled mint, lapacho plant, lavender, lemon grass, marjoram, mallow, balm, mistletoe, peppermint, marigold, rosemary, gentian, milfoil, thyme, hyssop, cinnamon etc.

"Fruit components" should be understood to mean in particular: fruit extracts, preferably from apples, bananas, pears, pineapple, oranges, grapefruit, cherry, sour cherry, limes, lemons, passion fruit, peaches, sea buckthorn, raspberries, strawberries, blackberries, redcurrants, gooseberries, kiwi fruits etc.

Preferably, it is provided for the mixed beer beverage to contain natural or nature-identical odour-bearing substances and/or flavourings as aroma components, such as essential oils from plants or fruit such as citrus oil, peppermint oil or clove oil, fruit essences, aroma-conferring fruit juices, anise, menthol, eucalyptus etc.

The colorant components are preferably colorants of plant origin such as carotinoids, flavonoids or anthocyanins, colorants of animal origin, inorganic pigments such as iron oxide pigments, products from enzymatic and non-

enzymatic browning, products formed by heating such as caramel, sugar colour or synthetic colorants such as azo compounds, triphenylmethane compounds, indigoid compounds, xanthene compounds or quinoline compounds. Suitable synthetic colorants are for example erythrosine, indigo carmine or tartrazine which are used for colour correction and/or for producing a pleasing appearance of the mixed beer beverage according to the invention.

The amino acid components are preferably mixtures of essential amino acids. Preferred amino acids are his, ile, leu, lys, thr, trp, val and taurin.

The acid components are preferably culinary acids. In a preferred embodiment, the beverages according to the invention are available as carbonated drinks, in other words, they may contain carbonic acid/carbon dioxide.

In a particularly preferred embodiment, the mixed beer beverages according to the invention also contain caffeine components such as extracts, preparations or essences from coffee beans, tea plant or parts thereof, maté tea plant or parts thereof, cola nut, cocoa bean or guarana.

#### Practical examples

The invention will be explained in further detail by the following examples.

The figures show:

Figure 1: Isomaltulose concentrations before and after incubation with organisms harmful to beer;

Figure 2: Isomaltulose concentration as a function of different stability factors;

Figure 3: Isomaltulose concentration in samples incubated with *S. cerevisiae* MJJ 2;

Figure 4: Sugar spectrum analysis after seven day incubation;

Figure 5: Acid concentration as a function of the micro-organisms selected;

Figure 6: Taste assessment of mixed beer beverages made from basic beer and lemonade (ideal taste note: 3): basic beer = Pilsen (Figure 6a), basic beer = dietetic beer (Figure 6b), basic beer = alcohol-free Pilsen (Figure 6d), basic beer = Doppelbock (Figure 6c).

Figure 7: Proportion of aroma components after fermentation of real worts.

Figure 8: Taste assessment of beers made from real worts.

Figure 9: Isomaltulose content after fermentation of model media. The range of values of 5 % above and below the initial value is highlighted.

Figure 10: Isomaltulose content after fermentation of model media with bacteria. The value range of 5 % above and below the initial value is highlighted.

Figure 11: Turbidity development in contaminated mixed beer beverages with sweetening agents sucrose (Suc), isomaltulose (Pal) or sweetener mixture (Sm) at angles of measurement of 90° and 25°.

Figure 12: Swelling of PET bottles with contaminated mixed beer beverages with the sweeteners sucrose (Suc), Isomaltulose (Pal) or sweetener mixture (Sm).

### Example 1: Metabolisation of isomaltulose in the model medium

#### 1.1 Model medium

The model medium was prepared as follows: 50 g of isomaltulose were dissolved in 500 ml of doubly distilled water; 6.7 g of yeast nitrogen base (YNB) were dissolved in 500 ml of doubly distilled water; 5 ml of the isomaltulose solution were autoclaved in test tubes with Durham tubes; the YNB solution was autoclaved individually, subsequently 5 ml each were pipetted into the autoclaved test tubes that had already been filled with 5 ml of isomaltulose solution.

The parameters of pH value, alcohol content and absence of oxygen were adjusted in a first batch such as they are present in the bottles/racked beer: 5 %

alcohol content (ethanol), pH value 4.5 and oxygen-free incubation (anaerobic vessel). In further batches, the growth-inhibiting factors were varied in each case, compare table 1.

Table 1:

| Factor                                     | Set values                    |
|--------------------------------------------|-------------------------------|
| pH                                         | 3.6; 3.8; 4.0; 4.2; 4.4       |
| Content of bitter compounds (isohumulones) | 10; 20; 30; 40; 50 mg/l       |
| Alcohol content                            | 4.5; 5; 5.5; 6; 6.5 % by vol. |

The untreated model medium has a pH value of 5.1. Using 100 ml of the model solution, the quantities of 0.1 normal sulphuric acid were determined as being 0.05 mole/l which were required to adjust the necessary pH values. Sulphuric acid was chosen so as not to supply an additional source of C to the medium.

A 20 % isohumulone solution was diluted in a ratio of 1:20 such that 10 mg of the solution contained approximately 0.1 mg of isohumulones:

20 % solution:      1 mg of the solution contains 0.2 mg of isohumulones  
                            10 mg of the solution contain 2 mg of isohumulones;

Dilution 1:20:      10 mg of the diluted solution contains 0.1 mg of  
                            isohumulones; 10 mg of the solution (0.1 mg of  
                            isohumulones) added to 10 ml of the model solution  
                            correspond to 10 mg isohumulones per litre.

The alcohol content was adjusted to the respective concentrations using 96 % non-denatured alcohol.

The test tubes incubated in an aerobic atmosphere were closed with a cotton wool stopper, anaerobic samples were also closed with cotton wool stoppers but incubated in an anaerobic vessel.

## 1.2 Micro-organisms

A group of micro-organisms was selected which are either known to be harmful to beer or exhibited the ability in preliminary tests of utilising isomaltulose. The micro-organisms are shown in Table 2.

Table 2:

| Micro-organisms                       | Beverage spoilage potential                                                                      |
|---------------------------------------|--------------------------------------------------------------------------------------------------|
| <i>Pediococcus damnosus</i>           | Anaerobic, grows in the presence of alcohol and hop bitter compounds                             |
| <i>Lactobacillus brevis</i>           | Anaerobic, grows in the presence of alcohol and hop bitter compounds; very frequent beer spoiler |
| <i>Megasphaera cerevisiae</i>         | Highly anaerobic, grows in the presence of alcohol and hop bitter compounds                      |
| <i>Pectinatus frisingensis</i>        | Highly anaerobic, grows in the presence of alcohol and hop bitter compounds                      |
| <i>Schizosaccharomyces pombe</i>      | Ferments isomaltulose rapidly and completely, apart from other sugars                            |
| <i>Saccharomyces diastaticus</i>      | Capable of fermenting dextrins (so-called superfermentors)                                       |
| <i>Saccharomyces cerevisiae</i> MJJ 2 | Ferments isomaltulose rapidly and completely, apart from other sugars                            |

The micro-organisms were cultivated in 100 ml of nutrient broth, subsequently washed with isomaltulose solution and resuspended in 5 ml of isomaltulose solution. The test tubes were incubated with 0.5 ml of the resuspended solution. Incubation took place at 26 °C.

### 1.3 Analyses

The occurrence of turbidity and gas formation was evaluated in the test tubes as an indicator of growth. In order to determine the actual isomaltulose decomposition, the extract was measured before and after incubation and the content of reducing sugars was determined by extinction measurement during the reaction with dinitrosalicylic acid (DNS) before and after incubation.

The extract measurement of the solution was effected by density determination using an U-tube densimeter (Anton Paar).

The content of isomaltulose was determined by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This produces a colour change in the solution from yellow to reddish brown. The concentration of a reducing sugar can be determined quantitatively by photometry on the basis of this colour change. Since isomaltulose is the only sugar in the test medium (in contrast to real media such as wort), it is possible by means of the DNS method to determine the concentration of isomaltulose before and after incubation.

30 g of K-Na tartrate were dissolved in 50 ml of distilled water and 20 ml of NaOH (2 mole/l) were added. 1 g of dinitrosalicylic acid (DNS) was introduced into the solution with stirring. Subsequently, it was made up with distilled water to 100 ml.

0.25 ml of the solution to be examined were placed into a test tube and mixed with 0.25 ml of the DNS solution. Both solutions were heated together for five minutes in a boiling water bath. After cooling, 9 ml of (distilled) water were added and after mixing, the absorption at 546 nm was measured. After adjusting a straight calibration line it was thus possible to determine the concentration of isomaltulose in the model solution before and after incubation.

It was found that a straight calibration line determined had the best accuracy in a concentration range of less than 1.5 % sugar in the solution. Correspondingly, the samples to be measured were diluted before the measurement in a ratio of 1:5 in order to reach the area of the best possible accuracy with a theoretical 5 % of isomaltulose (before incubation) in the solution.

## 1.4 Results

Table 3 shows the results of the visual growth assessment on the basis of the parameters of turbidity development and gas formation (n.o.: not observed, +: turbidity and/or gas formation, -: no turbidity or gas formation).

Table 3:

|                                | pH 4,5 | alcohol<br>5 %<br>vol./vol. | anaerobic |
|--------------------------------|--------|-----------------------------|-----------|
| Schizosaccharomyces pombe      | +      | +                           | +         |
| Saccharomyces cerevisiae MJJ 2 | +      | +                           | +         |
| Saccharomyces diastaticus      | +      | +                           | +         |
| Lactobacillus brevis           | -      | -                           | -         |
| Pediococcus damnosus           | -      | -                           | -         |
| Pectinatus frisingensis        | n.o.   | n.o.                        | +         |
| Megasphaera cerevisiae         | n.o.   | n.o.                        | +         |

Assessed according to the criteria of turbidity development and gas formation, growth occurred with all yeasts under all conditions. However, in the case of *Saccharomyces diastaticus*, only the turbidity development was observed without accompanying gas formation. This is unusual for a fermenting yeast. *Pectinatus frisingensis* and *Megasphaera cerevisiae* were able to develop turbidity under anaerobic conditions, and in the case of *Lactobacillus brevis* and *Pediococcus damnosus*, no signs of growth developed.

The parameters of turbidity development and gas development were documented visually over the incubation period. The results observed are illustrated as follows:

*1.4.1 Pediococcus brevis, Lactobacillus brevis, Megaspera cerevisiae, Pectinatus frisingensis*

In none of the inoculated batches was any turbidity development or any formation of gas observed.

*1.4.2 Schizosaccharomyces pombe*

After only a few days, the formation of gas was observed in some batches. However, judging by the concentrations of the different parameters, no regularity could be observed. After one week's incubation, the maximum amount of gas to be detected by the Durham tubes had been formed throughout, and in some batches, the yeast had begun to sediment. After ten days, the yeast had begun to sediment in all the batches.

*1.4.3 Saccharomyces diastaticus*

This yeast formed a slight turbidity in the course of the first week which increased in intensity in the second week. Obviously, the formation of turbidity was delayed by the alcohol concentrations of 6 % and 6.5 %. Similarly, at the pH value of 3.6, a developing turbidity was noted only three days after the formation of turbidity was observed at higher pH values. It appeared remarkable that large parts of the forming cell mass settled on the surface of the liquid. This applied both to the aerobically and anaerobically incubated batches. In none of the batches did the yeast form gas within three weeks. In the course of the second and third week, the yeast had sedimented completely except for the cell substance adhering to the glass at the surface of the liquid.

The observations under the microscope of both the yeast cells present at the edge and those sedimented at the bottom shows a large proportion of unusually small cells. After applying a smear of harvested small cells onto wort agar, cells of normal size were again observed. The conclusion can be drawn from this that *Saccharomyces diastaticus* has a tendency towards stunted growth in the case of isomaltulose present as only available source of carbohydrate.

#### 1.4.4 *Saccharomyces cerevisiae* MJJ 2

The addition of alcohols in a concentration of 6.5 % delays the formation of turbidity up to the beginning of the second week, the formation of gas arose only by the middle of the second week. In lower concentrations, the alcohol obviously did not inhibit the growth of the yeast. Reducing the pH value also remained without growth-inhibiting effect up to a value of 3.8. At a pH value of 3.6, gas and turbidity developed only by the middle of the second week. By adding hops, the development of turbidity and the formation of gas was also delayed until into the second incubation week. The formation of gas seems to be lower than in the other test series. In the batches incubated anaerobically, the growth activity developed more slowly than in the samples incubated aerobically. After the yeast had sedimented in the course of the third week, the volume of gas formed remained lower than that of the aerobic samples.

#### 1.4.5 Overall consideration

In Figure 1, the isomaltulose concentrations without incubation and those after three weeks' anaerobic incubation without preservation factors, determined by DNS assay, are illustrated for all the batches (anaerobic incubation, without preservation factors, three weeks incubation at 26 °C).

With the exception of the batches incubated with *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* MJJ 2, all the values are within a range of variation of less than 5 % around the measured concentration of the model solution without incubation.

This is interpreted to mean that only the yeasts *Saccharomyces cerevisiae* MJJ 2 and *Schizosaccharomyces pombe* were capable of degrading isomaltulose in an anaerobic atmosphere without added preservation factors within the period under consideration. *Saccharomyces cerevisiae* MJJ 2 reduced the isomaltulose content in the model solution by 26 %, whereas *Schizosaccharomyces pombe* was able to completely metabolise isomaltulose.

The bacteria investigated were incubated only anaerobically, a degradation of isomaltulose was not detected in any batch. The yeasts were also incubated

aerobically. Under aerobic conditions, *Schizosaccharomyces pombe* metabolises isomaltulose completely under all preservation parameters and in all concentrations.

Figure 2 shows isomaltulose concentrations measured by the DNS method in the samples incubated with *Saccharomyces diastaticus* (mean values of the series after three weeks incubation at 26 °C). The results are illustrated as mean values of the different preservation factors.

*Saccharomyces diastaticus* exhibited the ability of metabolising isomaltulose also under aerobic conditions. In this series of measurement, deviations in the measured concentration occurred which do not fit into a range of errors of 5 %. In the case of measured concentrations of more than 5 % isomaltulose, it is assumed here that lower proportions of water have evaporated from the solution in the course of the incubation time. For this reason, these measured values should be considered only as a trend.

Figure 3 shows isomaltulose concentrations measured by the DNS method in the samples incubated with *Saccharomyces cerevisiae* MJJ 2 (mean values of the selective factor series, aerobically incubated, in comparison with anaerobic incubation at 26 °C).

*Saccharomyces cerevisiae* MJJ 2 exhibited a better isomaltulose utilisation in an aerobic atmosphere than in the absence of oxygen. It can be clearly recognised in the figure that the variation of the pH value and the addition of alcohol within the scale examined here are not capable of inhibiting this yeast during the metabolisation of isomaltulose. However, the isomaltulose utilisation is obviously rendered more difficult for *Saccharomyces cerevisiae* MJJ 2 in the presence of hop bitter compounds and in the absence of oxygen.

#### Example 2: Commercial dietetic beer to which isomaltulose has been added

Isomaltulose was added to commercial dietetic beer (Henninger Diät-Pils) in a quantity such that the content of the real residual extract was 4 % wt./wt. In this beer, all beer-inherent selective factors are present in combination. The isomaltulose-containing dietetic beer was then incubated with the test micro-

organisms (Example 1, Table 2) for seven days at 26 °C. The test series was accompanied by analysis, by analyzing the worts and the beers according to the rules for methods of analysis for brewing technology of the Mitteleuropäischen Analysenkommission e.V. (Central European analytical committee) (MEBAK) and the saccharide spectra were measured additionally. The isomaltulose utilisation was determined by analysing the saccharide spectrum and acid spectrum since further reducing sugars, apart from isomaltulose, occur in the actual solution of beer. Before the analysis, the test micro-organisms were separated off by membrane filtration (0.45 µm) in order to produce a stable state. Measuring the acid spectrum and saccharide spectrum was effected by the HPLC/GC method in a manner known per se.

In Table 4, it is shown in which batches growth was observed on the basis of formation of turbidity (- = no turbidity, + = turbidity, ++ = strong turbidity).

Table 4:

| dietetic beer plus isomaltulose,<br>inoculated with: | turbidity (after 7 days) |
|------------------------------------------------------|--------------------------|
| <i>Saccharomyces diastaticus</i>                     | +                        |
| <i>Saccharomyces cerevisiae</i> MJJ 2                | ++                       |
| <i>Schizosaccharomyces pombe</i>                     | +                        |
| <i>Pectinatus frisingensis</i>                       | ++                       |
| <i>Pediococcus damnosus</i>                          | -                        |
| <i>Megasphaera cerevisiae</i>                        | ++                       |
| <i>Lactobacillus brevis</i>                          | -                        |

Within the period observed, *Pediococcus damnosus* and *Lactobacillus brevis* did not exhibit any growth (turbidity of the sample), *Schizosaccharomyces pombe* and *Saccharomyces diastaticus* produced only slight turbidity. *Saccharomyces cerevisiae* MJJ 2, *Pectinatus frisingensis* and *Megasphaera cerevisiae* were capable of developing strong turbidities.

Figure 4 shows the results of the sugar spectrum analysis of the dietetic beers after seven days' incubation at 26 °C.

In the zero beer (= commercial dietetic beer without addition of isomaltulose), none of the analysed sugars was found. Consequently, no utilisable low-molecular sugars are present therein. In the non-incubated dietetic beer to which isomaltulose had been added, fructose and glucose were found merely in trace, apart from 22.5 g/l of isomaltulose. In the incubated beers, with the exception of the beer incubated with *Schizosaccharomyces pombe*, the same isomaltulose concentrations as in the non-incubated beer were found within the range of the accuracy of measurement and weighing (deviations < 5 %). Measurable concentrations of glucose and fructose were formed again only in the beer incubated with *Schizosaccharomyces pombe*. In Table 5, the isomaltulose concentrations of the individual beers and the corresponding ratio to the initial concentration are illustrated.

Table 5:

| Sample                                                   | Isomaltulose concentration after incubation ( %) |
|----------------------------------------------------------|--------------------------------------------------|
| Commercial dietetic beer, unchanged                      | 0                                                |
| Commercial dietetic beer + isomaltulose (= H+P)          | 100                                              |
| H+P incubated with <i>Saccharomyces diastaticus</i>      | 100                                              |
| H+P incubated with <i>Saccharomyces cerevisiae</i> MJJ 2 | 100                                              |
| H+P incubated with <i>Schizosaccharomyces pombe</i>      | 83                                               |
| H+P incubated with <i>Pectinatus frisingensis</i>        | 100                                              |
| H+P incubated with <i>Pediococcus damnosus</i>           | 100                                              |
| H+P incubated with <i>Megasphaera cerevisiae</i>         | 100                                              |
| H+P incubated with <i>Lactobacillus brevis</i>           | 100                                              |

After separating off the micro-organisms by membrane filtration, the initial concentration of isomaltulose was still present in all the inoculated beers. The

only exception was the beer which had been incubated with *Schizosaccharomyces pombe*. In this case, a decrease by 17 % occurred and measurable concentrations of glucose and fructose, the cleavage products of isomaltulose, were again found only in this case. Consequently, the cell growth detected on the basis of the development of turbidity occurred only in the case of the yeast *Schizosaccharomyces pombe* on the basis of isomaltulose.

In addition, the pH values of the incubated beers were measured. All the values were 4.5 or 4.6. This result together with the analysis of the acid spectrum of the samples shows that no acid was formed by any of the micro-organisms incubated in the samples and confirms additionally in particular for the *Lactobacilli* that no growth had occurred. An important factor in the case of this spoilage of beverages by *Lactobacilli* is the formation of lactic acid which is capable of strongly impairing the aroma of beverage concerned.

In Figure 5, the results of the acid spectrum analysis are to be found. The contents of the measured acids are not within the region of abnormally high concentrations. Only in the samples incubated with *Pectinatus frisingensis* slightly elevated contents of succinic and acetic acid were found in comparison with the non-incubated samples. The values, being 0.3 g/l, are low. With respect to the content of lactic acid (lactate), it deserves to be mentioned that this has decreased in the samples incubated with *Pectinatus frisingensis*, *Pediococcus damnosus* and *Megasphaera cerevisiae* in comparison with the zero sample. Obviously, the lactate was metabolised as a result of the lack of utilisable substrate, a process described in the literature, in the course of which lactate is utilised as substrate for gluconeogenesis. In the sample incubated with *Lactobacillus brevis*, the same content was measured as in the non-incubated sample. Consequently, neither lactate was metabolised nor was it newly formed by the metabolism of the bacterium. This is taken as a further indication of the lack of metabolic activity of this bacterium.

### Example 3: Production of "dietetic beer"

"Dietetic beer" should be understood to mean beer in the case of which, ideally, only isomaltulose is present as carbohydrate in the finished product.

Since it is of decisive importance when producing dietetic beer to mash intensively, a mashing process was used as an example which strongly emphasises the temperature optimums of the starch-degrading enzymes of the malt:

- mashing in at 50 °C, 15 min rest
- heating to 55 °C (1 °C/min), 30 min rest
- heating to 62 °C (1 °C/min), 45 min rest
- heating to 65 °C (1 °C/min), 45 min rest
- heating to 68 °C (1 °C/min), 30 min rest
- heating to 70 °C (1 °C/min), 30 min rest
- heating to 72 °C (1 °C/min), 20 min rest
- heating to 78 °C (1 °C/min)
- mashing off

The malt grist consisted, in a proportion of 100 %, of Pilsen malt, hops were added in a dose of 90 mg  $\alpha$ -acid/l finished wort, consisting of CO<sub>2</sub> extract.

One part of it was produced as a reference example without the addition of isomaltulose and tasted and analysed. This beer represents the "zero beer". To another part of the beer, isomaltulose was added during boiling as a result of which the isomaltulose was present in the beer during the main fermentation process.

The main fermentation took place without pressure using the top-fermenting yeast *Saccharomyces carlsbergensis* MJJ 11, at 15 to 19 °C. Towards the end of fermentation, the temperature was raised in order to degrade the extract content of the wort as far as possible. The hose transfer took place after reaching the predetermined attenuation limit.

The test series was accompanied by analyses by which the worts and beers were analysed according to the methods of analysis for brewing technology of the Mitteleuropäischen Analysenkommission e.V. (MEBAK) and the saccharide spectra were additionally measured. With respect to the aroma of the beers, the contents of selected aroma-active esters and higher alcohols as well as acetaldehyde were measured. Moreover, the acid contents of the finished beers

were determined analytically and the finished beers subjected to a taste assessment both in comparison with each other and as an evaluation.

No impairment of the sensory characteristics such as quality of the aroma, quality of the taste, palativeness, liveliness and quality of the bitter taste (bitterness) came to light in the dietetic beer produced according to the invention. In many cases, the dietetic beer according to the invention was preferred to known dietetic beer (Henninger Diät-Pils in this case).

#### Example 4: Production of "beer with reduced alcohol content"

Parts of the original wort are replaced by isomaltulose such that less alcohol is formed during "normal" fermentation compared with a conventional full beer. During the process described above in example 3, mashing was carried out in such a way that as high a degree of fermentation as possible could be achieved. A high alcohol content is naturally obtained by complete fermentation of the available carbohydrates. For this reason, a mashing programme was used in this example which is shorter and less intensive and consequently does not completely convert the carbohydrate of the malt into fermentable sugars:

- mashing in at 62 °C; no rest
- heating to 66 °C (1 °C/min), 30 min rest
- heating to 72 °C (1 °C/min), 20 min rest
- heating to 78 °C
- mashing off

By replacing approximately one quarter of the original wort by isomaltulose at the time of boiling, the substrate available to the yeast is to be reduced.

Consequently, a beer can be produced which, compared with conventional full beers, contains less alcohol. As in the case of dietetic beers, the addition of hops takes place to 90 mg  $\alpha$ -acid per litre of finished wort.

The main fermentation process took place without pressure using the bottom-fermenting yeast *Saccharomyces carlsbergensis* MJJ 11, at 12 °C. The hose transfer took place with an extract content which was approximately 1.5 % above the expected extract on final fermentation.

The accompanying analysis (according to MEBAK and aroma-specific) corresponded to Example 3.

As shown in Example 3, the alcohol-free beer produced according to the invention exhibits no impairment of the sensory characteristics. In many cases, the alcohol-free beer according to the invention was preferred to known alcohol-free beer.

#### Example 5: Production of "malted drink"

Since a malted drink was to be produced with only a highly incomplete fermentation, it was not necessary to use a mashing programme for as high a degree of fermentation as possible. The same mashing programme was used as for example 4.

In order to adjust the colour of the product to as dark as is common for Malzbier, the following malt mixture was used:

- 40 % Pilsen malt
- 30 % Munich malt
- 20 % Cara malt
- 10 % colour malt

The original wort was adjusted in such a way that half of the extract was obtained from the malt whereas the other half was added in the form of isomaltulose (crystal sugar) to the boil. In total, a 12 % original wort was adjusted. The addition of hops took place to 15 mg  $\alpha$ -acid/l finished wort.

After separating off the hot break, the malted drink was filled into a keg and, after cooling, a little yeast (*Saccharomyces carlsbergensis* MJJ 11) was added. After a contact time of half a day at 0 °C, the yeast was largely separated off by transferring it under pressure to another storage vessel. This type of fermentation is modelled on the so-called cold contact process. After two weeks storage, filtration and bottling/racking took place.

The malted drinks thus obtained were assessed both from the sensory point of view and analytically (according to MEBAK and aroma-specific) as in Example 3 and 4 and compared with commercial maltbeer.

As in Examples 3 and 4, the malted drink produced according to the invention also showed no impairment of the sensory characteristics. In many cases, the malted drink according to the invention was preferred to known, commercial Malzbier.

#### Example 6: Production of mixed beer beverages

##### 6.1 Batches

Twelve different mixed beer beverages were made from beer components and lemonade components on a 10 l scale. In doing so, the beer component and the sweetener in the lemonade part were varied.

For the experiments, the following four different types of beer were selected (also with respect to subsequent tests of the biological stability of the beverages):

- a Pilsen beer in order to simulate commercial mixed beer beverages.
- a dietetic beer with a very low content of residual carbohydrates. Due to the fact that inherent utilisable carbohydrates are largely absent, the sweetener of the lemonade part used becomes the decisive factor regarding palatibility. Moreover, there is no beer-inherent substrate in the case of possible contamination.
- an alcohol-free Pilsen. The mixed beer beverage produced therewith does not contain the selective factor of alcohol, the influence of alcohol on the palatibility of the mixed beer beverage is also missing.
- a Doppelbock. In comparison with full beers, this has an elevated alcohol content and, on the other hand, an elevated residual extract

content which, in addition to the sweetener from the lemonade, presents a substrate for possible contaminants.

The lemonade part was made from a flavour system with lemon-lime aroma from Wild, item no. 3-110050331 and citric acid. The following were used as sweeteners:

- sucrose,
- isomaltulose,
- sweetener mixture from Wild (Sweet Up; consisting of cyclamate, saccharin, aspartame and acesulfame K).

The sweetener mixture was chosen since this product is used on an industrial scale for the production of mixed beer beverages and is consequently a practical product. Moreover, as a result of the mixture of the sweeteners, taste disadvantages of the individual components are compensated for. Citric acid is used for acidification.

The weighed ingredients were brought to solution in brewing liquor.

The variation of the different sweeteners and the beers gave the following test batches of mixed beer beverages:

Table 6:

12 experimental batches for beer-lemonade mixed drinks

|                     |              |                 |              |
|---------------------|--------------|-----------------|--------------|
| Pilsen              | Isomaltulose | Dietetic Pilsen | Isomaltulose |
|                     | Sucrose      |                 | Sucrose      |
|                     | Sweetener    |                 | Sweetener    |
| Alcohol-free Pilsen | Isomaltulose | Doppelbock      | Isomaltulose |
|                     | Sucrose      |                 | Sucrose      |
|                     | Sweetener    |                 | Sweetener    |

Sucrose and sweetener mixture were metered in a manner known per se, and corresponded to the recommendations of Wild Flavours. The isomaltulose was metered by adjusting, after tasting, the same sweetening strength as in the other lemonades. Beer and lemonade together were filled into a 30 l keg, mixed and subsequently carbonated. Carbonation took place by introducing CO<sub>2</sub> through the liquid channel of the dispensing head. To bond CO<sub>2</sub>, the kegs subjected to a gas pressure of 1.8 bar were stored at 0 °C for 24 hours. The keg pressure after cold storage was 0.8 - 0.9 bar.

## 6.2 Evaluation

Triangular taste assessments with preferences (according to MEBAK) were carried out with 10 tasters. Using the same basic beer, the variants with different sweeteners were tasted in comparison with each other. This had the purpose of finding out which sweetener was preferred from the sensory point of view. The deviating sample was to be determined and the respective preference recorded. The preference was taken into account only in the case of the right allocation of the deviating sample.

In addition, taste assessments were carried out with the mixed beer beverages produced. A separate plan was used for this purpose. By way of the assessment, a differentiation of the taste impressions was to be attempted as a function of the different sweeteners in the mixed beer beverage. Of decisive importance was the sweetness taste impression which, like the other characteristics, could be assessed positively as well as negatively. Apart from the sweetness, the following assessment criteria were available:

Palatefulness, bitterness, fruitiness, acidity, harmony (sweet-acid ratio) and refreshment. The verdicts were divided into grades of 1 to 5, "3" representing the desired value. Values above were taste impressions which were too intense such as e.g. "too sweet". Values below "3" were taste impressions which were too weak such as e.g. "not sweet enough". Consequently, the taster also had the possibility of assessing even the quality of the individual criteria more accurately. The assessment of refreshment was graded only from 1 to 3, with 3 meaning "refreshing" and 1 "not refreshing". Finally, the overall quality was assessed: integers from 1 (= poorest result) to 5 (= best result)).

## 6.3 Results

### 6.3.1 Chemical technical analyses

In the following, the analyses of the individual components of the lemonades are presented.

Table 7:

Analyse of the water used to produce the lemonade part.

|                     |                           |       |
|---------------------|---------------------------|-------|
| Total hardness      | German hardness           | 0.5   |
| Calcium hardness    | German hardness           | 0     |
| Residual alkalinity | German hardness           | 0.924 |
| pH value            |                           | 6.7   |
| p value             |                           | 0     |
| m value             |                           | 0.33  |
| Conductivity        | $\mu\text{S} / \text{cm}$ | 47    |

After treatment, the water is characterised by a very low hardness which is ideally required for producing acidified mixed beer beverages.

Table 8:

Analysis of the basic beers used

|                   |           | Pilsen | Dietetic Pilsen | Alcohol-free Pilsen | Doppelbock |
|-------------------|-----------|--------|-----------------|---------------------|------------|
| Original wort     | % by wt.  | 11.23  | 9.32            | 5.27                | 18.65      |
| Alcohol content   | % by vol. | 5.02   | 4.83            | 0.41                | 8.46       |
| Extract, apparent | % by wt.  | 1.76   | 0.01            | 4.46                | 3.47       |
| pH value          |           | 4.35   | 4.56            | 4.35                | 4.72       |
| Bitterness units  | BU        | 31     | 19              | 26                  | 25         |

It can clearly be seen that, in contrast to Pilsen beer, dietetic beer contains considerably less apparent residual extract (carbohydrates), the alcohol-free beer less alcohol with a similar residual extract content and the Doppelbock both clearly more alcohol as well as residual extract. The dietetic beer contains fewer bitterness units which could have a negative effect in the case of contamination with micro-organisms harmful to beer.

The pH values measured were measured as being lowest in Pilsen and the alcohol-free Pilsen, the Doppelbock had the highest value.

The following tables give the analyses of the finished mixed beer beverages.

Table 9:

Analysis of the mixed beer beverages using Pilsen as basic beer.

|                   |           | Isomaltulose | Sucrose | Sweetener |
|-------------------|-----------|--------------|---------|-----------|
| Extract, apparent | % by wt.  | 11.20        | 4.91    | 0.97      |
| Alcohol           | % by vol. | 2.60         | 2.68    | 2.53      |
| pH value          |           | 3.39         | 3.39    | 3.41      |
| Bitterness units  | BU        | 15           | 15      | 15        |

Table 10:

Analysis of the mixed beer beverages with the dietetic beer as basic beer.

|                   |           | Isomaltulose | Sucrose | Sweetener |
|-------------------|-----------|--------------|---------|-----------|
| Extract, apparent | % by wt.  | 10.27        | 4.01    | 0.14      |
| Alcohol           | % by vol. | 2.52         | 2.54    | 2.49      |
| pH value          |           | 3.46         | 3.47    | 3.48      |
| Bitterness units  | BU        | 12           | 11      | 12        |

Table 11:

Analysis of the mixed beer beverages with the alcohol-free as basic beer.

|                   |           | Isomaltulose | Sucrose | Sweetener |
|-------------------|-----------|--------------|---------|-----------|
| Extract, apparent | % by wt.  | 12.53        | 6.26    | 2.40      |
| Alcohol           | % by vol. | 0.04         | 0.06    | 0.05      |
| pH value          |           | 3.49         | 3.52    | 3.52      |
| Bitterness units  | BU        | 13           | 13      | 13        |

Table 12:

Analysis of the mixed beer beverages with Doppelbock as basic beer.

|                   |           | Isomaltulose | Sucrose | Sweetener |
|-------------------|-----------|--------------|---------|-----------|
| Extract, apparent | % by wt.  | 12.55        | 6.26    | 2.41      |
| Alcohol           | % by vol. | 3.85         | 3.79    | 3.91      |
| pH value          |           | 3.81         | 3.82    | 3.81      |
| Bitterness units  | BU        | 12           | 11      | 12        |

The differences in the analytical values due to the different basic beers can clearly be seen. Due to the citric acid of the lemonade part, the pH values of the mixed beer beverages are all below those of the basic beers.

As a result of the dilution with lemonade, the bitterness units are approximately half as high as those of the basic beers. As a result of this "halving effect", the influence of the lower initial bitterness of the dietetic beer decreases, the bitterness values of the mixed beer beverages are all of a similar order of magnitude. The alcohol contents of the mixed beer beverages were measured in line with the basic beers; the beverages produced with the alcohol-free beer contain less than 0.1 vol. % of alcohol and should therefore be regarded as alcohol-free, like the basic beer. The beverages with the Pilsen or dietetic beer used have an alcohol content of approximately 2.5 and slightly less than 2.7 vol.

% respectively, and the value of the mixed beverages produced with Doppelbock is slightly less than 4 vol. %. This value resembles that of normal pure beer beverages. The extract contents measured also correspond to the basic beers, the beverages sweetened with isomaltulose having the highest extract content due to the increased addition in comparison with sucrose. The sucrose-containing beverages have the second highest extracts, the mixtures sweetened with the sweeteners the lowest. Overall, the beverages based on dietetic beer have the lowest extract content, followed by the mixed beverages with the Pilsen beer. Interestingly, the basic beers of Doppelbock and the alcohol-free beer caused approximately the same residual extract content, since the alcohol-free beer obviously still contained unfermented residual extract. The suitability for diabetics of the dietetic beer also leads to the mixed beverages with the sweetener mixture (almost no calorific value) and the isomaltulose (low glycemic index) being suitable for diabetics.

### 6.3.2 Taste assessment

In the tables, the results of the triple glass taste assessment allocation are illustrated in which the preference for the individual sweeteners was to be determined, the basic beer being the same.

The taste assessments were carried out by ten people in each case; since the three sweeteners were the subject matter of the test, a total of thirty taste assessments to be evaluated per basic beer resulted.

Table 13:

Triple glass taste assessment with allocation, mixed beer beverages with different basic beers (15 correct allocations required for a significance level of 95 % ( $\alpha = 0,05$ ))

|                        | Pilsen | Dietetic Pilsen | Alcohol-free Pilsen | Doppelbock |
|------------------------|--------|-----------------|---------------------|------------|
| Isomaltulose preferred | 5      | 8               | 8                   | 9          |
| Sucrose                | 2      | 5               | 9                   | 7          |

|                      |    |    |    |    |
|----------------------|----|----|----|----|
| preferred            |    |    |    |    |
| Sweeteners preferred | 8  | 4  | 2  | 1  |
| Correct              | 15 | 17 | 19 | 17 |
| alpha (0,05)         | 15 | 15 | 15 | 15 |

In the case of the mixed beer beverage with Pilsen as basic beer, 15 taste assessments were allocated correctly, the different mixed beer beverages consequently differ significantly, depending on the sweetener used. Of the 15 correct allocations, the sweetener-containing beverage was preferred 8 times, that containing isomaltulose 5 times. 2 tasters preferred the mixed beer beverage sweetened with sucrose with Pilsen as basic beer.

In the case of the mixed beer beverage with dietetic beer as basic beer, the isomaltulose-containing beverage was preferred most frequently, the sweetener-containing mixed beer beverage was least frequently found to be the best.

19 tasters of the beer beverage mixed with alcohol-free beer allocated the corresponding samples correctly in the triple glass taste assessment; consequently, 19 assessments were evaluated. When alcohol-free Pilsen was used as basic beer, sweetening by the two sugars was preferred similarly frequently, both being considered as substantially better than the sweetener mixture.

17 assessments of the beer beverage mixed with Bockbier were used for the evaluation. Sweetening with isomaltulose was found to be most agreeable, followed by sucrose. Only one taster found sweetening with sweeteners to be most agreeable in the case of this basic beer.

In sum, the sugars isomaltulose and sucrose were found to be the most agreeable method of sweetening about equally frequently in the case of the four different basic beers (isomaltulose was preferred slightly more frequently), the use of the sweetener mixture was clearly preferred most rarely.

#### 6.3.2.1 Aroma profile

In Figure 6, the results of the evaluating taste assessment are illustrated.

The aroma profiles of the different mixed beer beverages with Pilsen as basic beer (Figure 6a) resemble each other very closely. Differences worth noting can be seen in the case of the parameters of bitterness and acidity. In this case, the beverage that had been sweetened with sweeteners received values beyond the optimum. Sweetening with the sugars sucrose and isomaltulose obviously compensates for these taste impressions more effectively than the sweetener mixture.

The aroma profiles are similar also for mixed beer beverages with dietetic Pilsen as basic beer (Figure 6b). Again, the taste impressions of bitterness and acidity are most prevalent in the case of sweetening with sweeteners, these parameters being perceived as least intensive in the case of beverages sweetened with isomaltulose. The beer sweetened with sucrose received just about the poorest assessment regarding the parameter of palatfulness. Overall, the palatfulness was assessed substantially more poorly, as was to be expected, than in the case of the mixed beer beverages with Pilsen basic beer.

In the case of mixed beer beverages with alcohol-free Pilsen as basic beer (Figure 6d), there were hardly detected any differences worth mentioning in the aroma profile. The beer sweetened with isomaltulose received a slightly better assessment for the parameters of fruitiness, palatfulness and harmony. The acidity impression was perceived most strongly in the case of the use of sweeteners although the sweetness impression was strongest also in this case; in some cases, the sweetness impression was perceived as being too strong.

In the case of the results of the evaluating taste assessment of mixed beer beverages with Doppelbock as basic beer, it can be seen that the aroma profiles of the beverages with this basic beer differ quite considerably from those of the other basic beers. Both the palatfulness and harmony as well as the sweetness impression go beyond the ideal assessment mark of 3, i.e. they were assessed as being too pronounced or deviating from the ideal. The bitterness, on the other hand, was perceived clearly less strongly although the

basic beer possessed higher bitterness units than the dietetic beer, in comparison. The aroma impression of the Bockbier used obviously dominates over the lemonade part and causes the poorer assessment of the mixed beverage than in the case of the other basic beers.

#### 6.3.2.2 Refreshing effect and overall quality

In addition, the refreshing effect was assessed as one of the integral characteristics of a mixed beer beverage as well as the overall quality. The results of this assessment are shown in Table 14.

Table 14:

|                     |              | Refreshment effect | Sensory quality overall |
|---------------------|--------------|--------------------|-------------------------|
| Pilsen              | Isomaltulose | 2.5                | 3.75                    |
|                     | Sucrose      | 2.75               | 3.5                     |
|                     | Sweetener    | 2.75               | 3.75                    |
| Dietetic            | Isomaltulose | 2.75               | 3.75                    |
|                     | Sucrose      | 2.5                | 3.5                     |
|                     | Sweetener    | 2.5                | 2.75                    |
| Alcohol-free Pilsen | Isomaltulose | 2.4                | 3.8                     |
|                     | Sucrose      | 2.3                | 3.5                     |
|                     | Sweetener    | 2.2                | 3.5                     |
| Doppelbock          | Isomaltulose | 1.7                | 3.3                     |
|                     | Sucrose      | 2.1                | 3.4                     |
|                     | Sweetener    | 1.7                | 2.8                     |

The mixed beer beverages with Pilsen as basic beer were rated with the highest value in the case of this assessment parameter. The beverages based on Doppelbock, on the other hand, were assessed as being least refreshing. No clear verdict was given regarding the different sweeteners, isomaltulose having been assessed as least refreshing, for example, in the Pilsen mixed beverage but best in the case of dietetic mixed beer beverage. The assessments of the other sweeteners are similar.

As regards the assessment of the overall sensory quality, there is no clear cut trend. Among the four mixed beer beverages assessed as being best, those that had been sweetened with isomaltulose occur three times. The beverage assessed as being poorest is the one with dietetic beer as basic beer and with a sweetener mixture. In this beverage, only very small quantities of carbohydrates are present. Overall, the mixed beverages with Doppelbock as basic beer were assessed as poorest.

The average values for refreshment and total quality (Table 15) indicate that isomaltulose and sucrose were assessed as being clearly better in both cases than the sweetener mixture. Isomaltulose received a better assessment regarding the overall quality, whereas beverages sweetened with sucrose were found to be slightly more refreshing.

Table 15:

|              | Refreshment effect | Sensory quality overall |
|--------------|--------------------|-------------------------|
| Isomaltulose | 2.34               | 3.65                    |
| Sucrose      | 2.40               | 3.48                    |
| Sweetener    | 2.29               | 3.20                    |

Sweetening of the mixed beer beverages with sucrose and isomaltulose should be regarded as being approximately equivalent on the basis of the taste assessments carried out, the use of the sweetener mixture losing out clearly in comparison.

## 6.4 Contamination of the mixed beer beverages

### 6.4.1 Batches

The finished mixed beverages were filled by means of a manual filling facility into 0,5 l PET bottles. Before closing them with a screw cap, 50 µl of the

washed pure culture suspension were pipetted into the bottles in the stream of CO<sub>2</sub>.

The following bacteria were used for contamination:

- *Lactobacillus brevis* (DSM 20054)
- *Pediococcus damnosus* (DSM: 20331)
- and *Megasphaera cerevisiae* (wild strain)

In addition, some bottles were incubated with the following yeasts:

- *Saccharomyces diastaticus* (wild strain)
- *Saccharomyces cerevisiae* MJJ2
- and *Schizosaccharomyces pombe* (wild strain)

Incubation was effected at 20 °C with exclusion of light in closed bottles.

#### *6.4.2 Measuring the microbiological spoilage*

The turbidity in the bottles was measured with a process photometer (Sigrist KTL 30-21) at angles of measurement of 90° and 25°. The measurement at two different angles of measurement was carried out in order to take into account the different cell sizes of the yeasts and bacteria. The detectable turbidity maximum was at 20 EBC.

Swelling of the bottle was determined on the basis of the deformation at selected points of the PET bottles used:

- absolute height of the bottle
- diameter at a level of 11.5 cm
- and shoulder base

A digital slide gauge was used for the measurement.

#### *6.4.3 Turbidity developments*

After incubation of the different batches, the turbidity developments shown in the following illustrations were obtained.

#### 6.4.3.1 *S. diastaticus*

Figure 11a shows the turbidity developments of mixed beer beverage with the basic Pilsen beer, contaminated with *Saccharomyces diastaticus*. The beverage with the sucrose sweetening reaches the maximum turbidity value as early as on the second day of incubation. In the case of sweetening with sweeteners, the turbidity increases slowly in order to reach the maximum value also within the period under consideration. Obviously, *Saccharomyces diastaticus* is capable of growing on the basis of the residual extract of the basic beer and of rendering the beverage turbid. The turbidity in the isomaltulose-containing beverage increases most slowly; it can be assumed that the growth takes place essentially on the basis of the beer extract and isomaltulose does not accelerate the beverage becoming spoiled.

Figure 11b shows the turbidity developments of the mixed beer beverage with the dietetic Pilsen basic beer, contaminated with *Saccharomyces diastaticus*. When using the dietetic beer as basic beer, a clear turbidity arises only in the case of conventional sucrose sweetening. The dietetic beer does not contribute any extract of its own such that the conclusion can be drawn that *Saccharomyces diastaticus* is incapable of utilising either the sweetener mixture or the isomaltulose as substrate.

Figure 11c shows the turbidity developments of the mixed beer beverage with the alcohol-free Pilsen basic beer, contaminated with *Saccharomyces diastaticus*. In the case of the batches with the alcohol-free beer, all batches reach the turbidity maximum in the period considered. Sucrose produces the most rapid increase in turbidity, in the two other batches the yeast is capable of growing, unhampered by alcohol, as a result of the residual extract from the beer content. The slowest spoilage occurs in the case of the isomaltulose-containing batches.

Figure 11d shows the turbidity developments of the mixed beer beverage with the Doppelbock basic beer, contaminated with *Saccharomyces diastaticus*. In spite of the higher alcohol content introduced into the mixed beer beverage by using the Doppelbock, almost all batches reach the maximum turbidity value almost as quickly approximately on the third day. These mixed beverages

exhibit the highest pH values and relatively low contents of bitter compounds. The basic beer used, moreover, contained large quantities of fermentable residual extract on the basis of which cell growth occurred.

#### 6.4.3.2 *S. cerevisiae* MJJ2

Figure 11e shows the turbidity developments of the mixed beer beverage with the Pilsen basic beer, contaminated by *Saccharomyces cerevisiae* MJJ2. The top-fermenting brewer's yeast *Saccharomyces cerevisiae* MJJ2 developed the most rapid cell growth in the presence of sucrose. Considerably later, though undoubtedly on the basis of isomaltulose, the increase in turbidity occurs in the case of the isomaltulose batch. The turbidity which does not increase in the case of sweetener sweetening shows that neither the sweeteners nor the residual extract of the Pilsen beer can be utilised as a basis for growth.

Figure 11f shows the turbidity developments of the mixed beer beverage with the dietetic Pilsen basic beer, contaminated by *Saccharomyces cerevisiae*. In the case of mixed beer beverage produced on the basis of dietetic beer, the sucrose-containing beverage becomes turbid very rapidly. Isomaltulose is metabolised more slowly than sucrose but more rapidly than when using Pilsen beer as basic beer, this being essentially attributable to the fact that dietetic beer contains less hops and slightly less alcohol. These two substances inhibit the growth of yeast less strongly in this trial batch. In the batch with the sweeteners, no increase in turbidity occurs.

Figure 11g shows the turbidity developments of the mixed beer beverage with the alcohol-free Pilsen basic beer, contaminated with *Saccharomyces cerevisiae*. In the absence of alcohol, this yeast is capable of utilising at least part of the residual extract present in the beer, shown by the increase in turbidity in the case of sweetener sweetening. In the case of sucrose sweetening, the turbidity maximum is reached as early as on the third day. In the case of the isomaltulose-containing batch, the increase in turbidity takes place more slowly. In all batches, parts of the residual extract present in the beer are utilised.

Figure 11h shows the turbidity developments of the mixed beer beverage with the Doppelbock basic beer, contaminated with *Saccharomyces cerevisiae*. The yeast contemplated here, too, is capable of growing very rapidly in the case of all the batches with Doppelbock as basic beer. This is attributed to the large quantity of fermentable residual extract and the relatively low influences of the selective factors of pH value and content of bitter compounds.

#### 6.4.3.3 *S. pombe*

Figure 11i shows the turbidity developments of the mixed beer beverage with the Pilsen basic beer, contaminated with *Schizosaccharomyces pombe*. The batches with the Pilsen basic beer and the yeast *Schizosaccharomyces pombe* exhibit a highly non-typical development. Since the batches with the sweeteners on the one hand and sucrose on the other exhibit a considerable turbidity right from the beginning, but which remains almost constant over the entire period, the conclusion is drawn that a colloidal turbidity of the beer portion is present, possibly caused by an excessive absorption of oxygen during bottling/racking. This is supported by the fact that the values are high mainly in the case of the turbidity measurement of 25°. Measurements at 25° show a trend towards smaller turbidity particles, whereas yeast cells should be recognisable even at 90°.

Figure 11j shows the turbidity developments of the mixed beer beverage with the dietetic Pilsen basic beer, contaminated with *Schizosaccharomyces pombe*. During the incubation of the mixed beverage with the dietetic beer as beer portion using *Schizosaccharomyces pombe*, no marked increase in turbidity can be seen in any of the batches. This is assessed as being due to the yeast being prevented from growing by the combined effect of the exclusion of oxygen, presence of alcohol and hops. However, since these yeasts had been capable of utilising isomaltulose in a similar series of experiments in pure dietetic beer, the pH value reduced as a result of the lemonade probably plays a decisive part regarding the non-occurrence of cell growth in these batches. Table 10 shows that the pH values of the mixed beer beverages amount to 3.5 or even slightly less, whereas the pH value of a beer is slightly above 4.

Figure 11k shows the turbidity developments of the mixed beer beverage with the alcohol-free Pilsen basic beer, contaminated with *Schizosaccharomyces pombe*. In the absence of alcohol, *Schizosaccharomyces pombe* is obviously capable of developing a slight activity in spite of the low pH value. This applies at least to the conversion with sucrose. When the batches were sweetened with isomaltulose and sweeteners which are more difficult to utilise, only very slight and ambiguous increases in turbidity can be noted in the period under consideration.

Figure 11l shows the turbidity developments of the mixed beer beverage with the Doppelbock basic beer, contaminated with *Schizosaccharomyces pombe*. The beverages which have been produced with Doppelbock exhibit a fairly rapid increase in turbidity in the case of all batches. This is attributed to the fact that not only the above-mentioned high proportions of fermentable sugars are introduced from the beer, but also that these mixed beer beverages additionally have the highest pH values of all the batches (compare Table 12). These are just above 3.8.

#### 6.4.3.4 *P. damnosus*

Figure 11m shows the turbidity developments of the mixed beer beverage with the Pilsen basic beer, contaminated with *Pediococcus damnosus*. When using Pilsen beer, all the batches remained stable for a long period after contamination with *Pediococcus damnosus*. The batches sweetened with sucrose exhibited a marked turbidity and consequently spoilage by the end of the period under consideration.

Figure 11n shows the turbidity developments of the mixed beer beverage with the dietetic Pilsen basic beer, contaminated with *Pediococcus damnosus*. The turbidity developments detected when using the dietetic basic beer correlate well with those of the Pilsen basic beer. The more rapid increase in turbidity in the case of sucrose-containing batches is due to the slightly lower contents of alcohol and bitter compounds. The fact that, in the case of dietetic beer, which does not contribute any carbohydrate part of its own, an increase in turbidity was detectable only in the case of sweetening with sucrose, too, confirms the assumption made in the case of Pilsen basic beer that cell growth indeed took

place on the basis of the sucrose sweetener, the other sweeteners not providing any sustenance for *P. damnosus*. Figure 11o shows the turbidity development of the mixed beer beverage with the alcohol-free Pilsen basic beer, contaminated with *Pediococcus damnosus*.

In the case of the batches with the alcohol-free beer, too, only the batches with sucrose exhibit an increase in turbidity. *P. damnosus* is able to utilise only sucrose.

Figure 11p shows the turbidity developments of the mixed beer beverage with the Doppelbock basic beer, contaminated with *Pediococcus damnosus*. The formation of turbidity observed in the other batches is delayed here by the higher proportion of alcohol. However, towards the end of the period considered, a slight increase in turbidity arises in the case of all the batches. From this, the conclusion can be drawn that a slight growth takes place on the basis of the residual sugar of the beer portion.

#### 6.4.3.5 *L. brevis*

Figure 11q shows the turbidity developments of the mixed beer beverage with Pilsen basic beer, contaminated with *Lactobacillus brevis*. During the incubation of the batches with the Pilsen basic beer with *Lactobacillus brevis*, no increase in turbidity is observed. Constant, slightly elevated values are regarded as basic turbidity which may be the result, among other things, also of the introduction of the cell suspension during inoculation.

Figure 11r shows the turbidity developments of the mixed beer beverage with the dietetic Pilsen basic beer, contaminated with *Lactobacillus brevis*. All the batches considered remained constant for a prolonged period. A slight increase in turbidity can be seen in the batches with sucrose. This effect may be due to the slightly lower concentrations of alcohol and bitter compounds of the dietetic beer.

Figure 11s shows the turbidity developments of the mixed beer beverage with the alcohol-free Pilsen basic beer, contaminated with *Lactobacillus brevis*. In the batches with alcohol-free basic beer, the increase in turbidity observable in

the batch containing sucrose proves that *L. brevis* is capable, in the absence of alcohol, of spoiling sucrose-containing beverages. The two other batches remain constant; no utilisation of the isomaltulose or the sweetener takes place.

Figure 11t shows the turbidity developments of the mixed beer beverage with the Doppelbock basic beer, contaminated with *Lactobacillus brevis*. In the batches of the mixed beer beverages produced with Doppelbock as basic beer, no increase in turbidity can be seen. Presumably as a result of the higher alcohol content, together with the pH value which is lower than is typical for beer, no growth activity can be detected.

#### 6.4.3.6 *M. cerevisiae*

Figure 11u shows the turbidity developments of the mixed beer beverage with the Pilsen basic beer, contaminated with *Megasphaera cerevisiae*. The test series on the mixtures with Pilsen basic beer will be illustrated here exemplarily for all the batches with *Megasphaera cerevisiae* as a whole. In none of the batches has an increase in turbidity been observed. In all probability, this is due to the pH values which are markedly below 4 even in the case of the Doppelbock mixed beverage.

#### 6.4.4 Swelling of bottles

Just as clear cut a spoilage of a soft drink as turbidity is the so-called swelling or blowing of the bottles. If the bottle content is spoiled by microbial activity, deformations (swelling) on the bottle as a result of the increased internal pressure, as well as the turbidity formed can be observed.

The dimensions illustrated in Figures 12a to l of the non-incubated bottles (zero bottle) were measured after filling of the bottles but before incubation. This means that slight variations regarding this reference value are due to the normal pressure increase which arises as a result of storing the carbonated drink at 20 °C. In the following, this expansion will be called normal expansion. It is to be illustrated by way of the example of the following illustration.

Figure 12a shows the changes in height of the contaminated bottles following incubation with *Saccharomyces diastaticus* in comparison with the non-

deformed bottle (zero bottle). It can be seen that, following incubation of the filled bottles with *Megasphaera cerevisiae*, slight deviations in the filled bottles in comparison with the zero bottle arise. On the basis of the turbidity measured, however, no growth was determined. Moreover, it should be noted that *Megasphaera* forms only minimum quantities of CO<sub>2</sub>, if any at all, when growth occurs and thus is hardly capable of contributing to an increase in the internal pressure of the bottle.

The development of swelling is generally suitable rather as proof of beverage spoilage by yeasts than by bacteria since yeasts form markedly more CO<sub>2</sub> on metabolism activity. The danger posed by swelling is thus more relevant regarding spoilage by yeasts than by bacteria which spoil beverages rather by the formation of turbidity and off-flavours.

Figure 12b shows the changes in height of the contaminated bottles after incubation with *Saccharomyces diastaticus* in comparison with the non-deformed bottle (zero bottle). Essentially, the deformations of the bottle that have developed correlate with the developments determined already for the formation of turbidity. Slight variations in the dimensions may have been caused by an increase in the internal pressure which may arise as a result of storage of a carbonated beverage at 20 °C. Substantial changes in the absolute height of the bottles inoculated with *Saccharomyces diastaticus* can be observed only in the case of sucrose-containing batches.

Figure 12c shows the changes in the diameter of the contaminated bottles following incubation with *Saccharomyces diastaticus* in comparison with the non-deformed bottle (zero bottle). As in the case of the height measurements, marked changes in the bottle dimensions are recognisable only when *Saccharomyces diastaticus* was able to utilise sucrose.

Figure 12d shows the changes in the shoulder bases of the contaminated bottles following incubation with *Saccharomyces diastaticus* in comparison with the non-deformed bottle (zero bottle). Again, marked changes can be noted after incubation with *Saccharomyces diastaticus* only in the beverages sweetened with sucrose. Interestingly, the strongest deviations occur in the batch with the dietetic beer in which the carbohydrate portion originates solely

from the sweetening of the lemonade portion. In all the batches inoculated with *Saccharomyces diastaticus*, only sucrose was utilised as a basis for metabolism.

Figure 12e shows the changes in height of the contaminated bottles after incubation with *Saccharomyces cerevisiae* MJJ2 in comparison with the non-deformed bottle (zero bottle). For the batches incubated with *Saccharomyces cerevisiae* MJJ2, the strongest deviations occurred in the beverages sweetened with sucrose. The isomaltulose-containing beverages in which also more or less strong turbidities occurred as a result of cell multiplication, exhibit a slight height expansion, though a considerably lower one than in the case of sweetening with sucrose.

Figure 12f shows the changes in diameter of the contaminated bottles following incubation with *Saccharomyces cerevisiae* MJJ2 in comparison with the non deformed bottle (zero bottle). The same applies to the changes in diameter of the bottles following incubation with *Saccharomyces cerevisiae* MJJ2 as for the height expansion in that the most marked deformations occurred in the case of beverages sweetened with sucrose, while the expansions in the case of the isomaltulose-containing and sweetener-containing batches are in the region of normal expansion.

Figure 12g shows the changes in the shoulder base heights of the contaminated bottles following incubation with *Saccharomyces cerevisiae* MJJ2 in comparison with the non-deformed bottle (zero bottle). It can be clearly seen that the relevant deformation of the bottle regarding the parameters of height of the shoulder base of the bottles incubated with *Saccharomyces cerevisiae* MJJ2 occurred only in the trial batches sweetened with sucrose.

Figure 12h shows the changes in height of the contaminated bottles following incubation with *Schizosaccharomyces pombe* in comparison with the non-deformed bottle (zero bottle). In none of the batches incubated with *Schizosaccharomyces* does any notable growth occur, correspondingly, no expansions of the bottles exceeding normal expansion were detected. The same applies to Figure 12i and j.

Figure 12i shows the changes in diameter of the contaminated bottles following incubation with *Schizosaccharomyces pombe* in comparison with the non-deformed bottle (zero bottle). Figure 12j shows the changes in shoulder base heights of the contaminated bottles following incubation with *Schizosaccharomyces pombe* in comparison with the non-deformed bottle (zero bottle). In the trial batches contaminated with bacteria, no marked deformations have occurred in those cases, where no turbidity was measured, just as was expected. *Pediococcus damnosus* was capable (exception: Doppelbock as basic beer) of forming turbidity in the mixed beverages sweetened with sucrose. In the mixtures with dietetic beer and the alcohol-free beer, this occurred even substantially before the end of the period considered. Slight deviations in the height expansion can be detected.

Figure 12k shows the changes in height of the contaminated bottles following incubation with *Pediococcus damnosus* in comparison with the non-deformed bottle (zero bottle). This expansion is only slight and was not verified at the other measuring points. The reason for this may be that *Pediococcus* belongs to the homofermentative lactic acid bacteria which are capable of forming only small quantities of CO<sub>2</sub> even in the case of strong metabolic activity.

Figure 12l shows the changes in height of the contaminated bottles following incubation with *Lactobacillus brevis* in comparison with the non-deformed bottle (zero bottle). By way of the turbidity, the growth of *L. brevis* was detected only in the mixed beer beverage produced with alcohol-free beer and the lemonade portion sweetened with sucrose. Although *Lactobacillus brevis* is heterofermentative, the growth occurred only very slowly such that a deviation was detected only in the parameter of height expansion which was only slightly above the normal expansion.

## 6.5 Summary

Mixed beer beverages were produced which, because of the different basic beers selected, differed by the parameters of alcohol content, carbohydrate introduction of the beer portion and content of bitter compounds. By varying the sweetener, sucrose or isomaltulose was supplied to the mixed beverages as possible substrate or, by using sweeteners of the lemonade part no utilisable

carbohydrates were introduced. Because of the high relevance of yeasts as damaging substances in low-alcohol but sugar-rich soft drinks, the beverages were deliberately contaminated with three different yeasts and incubated at 20 °C. The yeast *Saccharomyces diastaticus* was selected because it is highly significant as a substance harmful to beer, since it is, apart from the normal ability of attenuating low-molecular carbohydrates, also capable of attenuating longer carbohydrates chains, the so-called dextrins. Moreover, the yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* MJJ2 were used. They were capable of utilising isomaltulose in preliminary tests; *Schizosaccharomyces pombe* was capable of doing this also in bottled beer.

Apart from these yeasts, bacteria were also used which have great significance as spoilage germs for bottled beer. *Pediococcus damnosus*, *Lactobacillus brevis* and *Megasphaera cerevisiae* were chosen.

It was found that the yeasts of *Saccharomyces diastaticus* and *Saccharomyces cerevisiae* MJJ2 were rapidly capable of spoiling most of the contaminated beverages. This occurred both by forming a strong turbidity and by a strong deformation of the bottles (swelling). But whereas *Saccharomyces diastaticus* was capable of growing in the trial batch with dietetic beer (introduction of carbohydrates only by the lemonade portion) only in the presence of sucrose, *Saccharomyces cerevisiae* MJJ2 also grew in the batch sweetened with isomaltulose, though surprisingly more slowly than in the case of sweetening with sucrose. From this, the conclusion is drawn that *Saccharomyces cerevisiae* MJJ2 was capable of utilising also isomaltulose under the circumstances concerned, though surprisingly more slowly than sucrose. Surprisingly, *Saccharomyces diastaticus* was not able to utilise isomaltulose in any of the beverages produced.

In general, this yeast is not capable of utilising isomaltulose as a substrate. The proven growth of *Saccharomyces* yeasts in the batches which had been produced with basic beers other than dietetic beer occurred in the case of all sweetening variants in an almost identical manner and is attributed to the utilisation of large quantities of residual extract which had been co-introduced by the beer portion into the finished mixed beverage.

In general, *Schizosaccharomyces pombe* did not exhibit any noticeable activity in any of the batches investigated. It is assumed that this is due above all to the pH values which, as a result of the lemonade admixture, are considerably below that of normal beers.

In the case of the evaluation of the trial batches inoculated with bacteria harmful to beer, it is striking that only those batches could be rendered turbid by *Pediococcus damnosus* and *Lactobacillus brevis* which had been sweetened with sucrose. In none of the batches sweetened with isomaltulose or sweetener mixture was it possible for these bacteria to cause turbidity. *Megasphera cerevisiae* which had also been investigated, was generally not able to grow in the mixed beer beverages, the reason for this should be sought above all in the low pH values of the beverages.

To some up, among the organisms investigated, only *Saccharomyces cerevisiae* MJJ2 is capable of utilising the isomaltulose used as sweetener, though surprisingly more slowly than the sucrose present alternatively. Surprisingly, all other organisms are incapable of utilising isomaltulose.

In contrast to the use of sucrose for sweetening the lemonade portion of mixed beer beverages, isomaltulose is also capable of substantially improving the biological stability. The batches with sweetener mixture have proved just as stable as the beverages sweetened according to the invention with isomaltulose. However, these show poorer tasting results which, on the whole, are unacceptable.

#### Example 7: Influence of isomaltulose on the aroma profile of fermented real worts

##### 7.1 Real worts

A Pilsen wort was produced. A part of this wort was treated in such a way that approximately one quarter of the extract consisted of isomaltulose. The untreated original wort and the isomaltulose-containing wort were fermented under the same conditions (without pressure, 12 °C) by the same yeasts as those used in the model worts.

Since normal practice of beer production was to be simulated, these worts were not used in one step up to final fermentation, but up to 1 to 1,5 % above the extract to be expected on final fermentation, subsequently a 14 day secondary fermentation was added on at 1 °C. The beers thus produced were analysed in a beer-specific manner according to MEBAK and by gas chromatography for the same aroma components chosen as for the model worts. In addition, taste assessments were carried out. The change in the analytically determined aroma profile as well as that of the taste impression as a function of the isomaltulose content was to be determined.

In the trial brewery of VLB, a beer wort (Pilsen type) was produced which was adjusted, by redilution with water and addition of isomaltulose, in such a way that approximately 25 % of the extract content of the wort consisted of isomaltulose. The unchanged wort and the isomaltulose-containing wort were fermented under the same conditions in parallel by different yeasts.

The wort analyses are given in Table 16.

Table 16:

Wort analyses with and without isomaltulose

| Parameter                                     | Unit | Original wort | Wort w/ 25 % isomaltulose |
|-----------------------------------------------|------|---------------|---------------------------|
| Extract content                               | %    | 11.26         | 11.33                     |
| Extract, apparent, final fermentation reached | %    | 1.93          | 4.2                       |
| Final fermentation, apparent                  | %    | 83.3          | 62.6                      |
| pH                                            |      | 5.36          | 5.2                       |
| Colour depth                                  | EBC  | 8.6           | 6.4                       |
| Bitterness units                              | BU   | 48.1          | 31.6                      |
| Total nitrogen                                | ppm  | 969           | 655                       |
| Free amino nitrogen                           | ppm  | 175           | 124                       |

|      |     |      |      |
|------|-----|------|------|
| Zinc | ppm | 0.17 | 0.15 |
| DMS  | ppb | 30   | 20   |

The worts were adjusted to a very similar extract content. The addition of isomaltulose caused the degree of fermentation to fall since the proportion of non-fermentable carbohydrates (in the analysis of final fermentation) increases as a result of the substitution of wort-inherent extract by isomaltulose. The other analytical values change in line with the redilution, i.e. the content such as bitter compounds or protein fractions, falls.

Both worts were fermented with the following four yeasts respectively:

- *Saccharomyces carlsbergensis* MJJ 11,
- *Saccharomyces cerevisiae* MJJ 25,
- *Saccharomyces cerevisiae* MJJ 2,
- *Schizosaccharomyces pombe*

## 7.2 Analysis of the aroma components

On completion of fermentation (= no decrease in extract for 4 days), the following aroma components were determined in all fermentation batches: acetaldehyde, ethyl acetate, 1-propanol, isobutanol, isoamyl acetate, 2-methylbutanol, 3-methylbutanol, 2-phenylethanol, phenyl acetate. Apart from the relevant aroma components, the vicinal diketones formed during fermentation were also determined. These are relatively important aroma components which, in many cases, are used in brewing practice as key substances for controlling main fermentation.

## 7.3 Results

### 7.3.1 Progression of fermentation

The following result is obtained regarding the progression of fermentation of the two real worts (with and without isomaltulose) during fermentation with *Saccharomyces carlsbergensis* MJJ 11: the decrease in extract is initially identical but then the curve of the decrease becomes flatter in the case of the isomaltulose-containing wort. Once the non-isomaltulose-containing wort had

reached the desired value, the fermentation of the isomaltulose-containing wort was broken off as well. As expected, the residual extract remains higher in this case.

In the case of fermentation with *Saccharomyces cerevisiae* MJJ 25, the curves of the extract decrease run parallel only initially. The curves are differentiated relatively early (as early as on day 5 of fermentation) and the difference between the remaining extracts is relatively marked.

Regarding the progression of fermentation of real wort (with and without isomaltulose) on fermentation with *Saccharomyces cerevisiae* MJJ 2, the following result is obtained: the progression of fermentation resembles that presented above, the decrease in extract begins in a similar manner, subsequently the curve of the isomaltulose-containing wort becomes flatter.

Regarding the progression of fermentation of real wort (with and without isomaltulose) on fermentation with *Schizosaccharomyces pombe*, the following result is obtained: the curves of the extract development are almost identical in the case of *Schizosaccharomyces pombe*. The end values obtained are also very similar since isomaltulose can also be utilised as substrate by this yeast.

### 7.3.2 Analysis of the finished beers

Table 17 shows the analytical values of worts fermented by *Saccharomyces carlsbergensis* MJJ 11 and *Saccharomyces cerevisiae* MJJ 25 (with and without isomaltulose)

Table 17:

|                                  | MJJ 11<br>without<br>isomaltulose | MJJ 11 with<br>isomaltulose | MJJ 25<br>without<br>isomaltulose | MJJ 25 with<br>isomaltulose |
|----------------------------------|-----------------------------------|-----------------------------|-----------------------------------|-----------------------------|
| Original wort,<br>calculated [%] | 11.25                             | 11.24                       | 11.32                             | 11.28                       |
| Extract, apparent,<br>[%]        | 2.35                              | 3.68                        | 3.3                               | 4.16                        |

|                       |      |      |      |      |
|-----------------------|------|------|------|------|
| Extract, real [%]     | 4.04 | 5.1  | 4.82 | 5.44 |
| Alcohol [% by vol.]   | 4.71 | 3.98 | 4.28 | 3.59 |
| pH                    | 4.4  | 4.25 | 4.67 | 4.4  |
| Bitterness units [BU] | 30   | 27   | 33   | 29   |
| Head retention [s]    | 276  | 263  | 343  | 281  |

Table 18 shows the analytical values of the worts fermented with *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* MJJ 2 (with and without isomaltulose)

Table 18:

|                               | Schiz. pombe without isomaltulose | Schiz. pombe with isomaltulose | MJJ 2 without isomaltulose | MJJ 2 with isomaltulose |
|-------------------------------|-----------------------------------|--------------------------------|----------------------------|-------------------------|
| Original wort, calculated [%] | 11.27                             | 11.32                          | 11.28                      | 11.56                   |
| Extract, apparent, [%]        | 2.41                              | 4.21                           | 2.13                       | 4.58                    |
| Extract, real [%]             | 4.08                              | 5.5                            | 3.87                       | 5.91                    |
| Alcohol [% by vol.]           | 4.67                              | 3.64                           | 4.86                       | 3.75                    |
| pH                            | 4.38                              | 4.36                           | 4.45                       | 4.36                    |
| Bitterness units [BU]         | 33                                | 26                             | 29                         | 25                      |
| Head retention [s]            | 276                               | 197                            | 228                        | 256                     |

Higher residual extracts after fermentation can be clearly seen in the case of beers to which isomaltulose had been added, since the isomaltulose was not fermented. This leads to a lower alcohol content in all cases. This also applies to *Schizosaccharomyces pombe* which is capable of utilising the isomaltulose-containing solutions in the model worts in a similarly satisfactory manner as the reference solution. In complex mixed carbohydrate solutions, other sugars are obviously utilised by this yeast first, too. Before it was possible to degrade isomaltulose, the fermentation had been terminated in this series of test. The

pH value of the isomaltulose-containing beers is slightly lower in all cases than that of the comparative beers; the same also applies to the bitterness units although, in this case, the lower values need to be attributed to redilution.

### *7.3.3 Aroma components*

Figure 7a shows the content of aroma components following fermentation of the real worts by *Saccharomyces carlsbergensis* MJJ 11 and *Saccharomyces cerevisiae* MJJ 25.

No clear-cut influence of the addition of isomaltulose on the formation of aroma components can be detected. Although MJJ 11 forms larger quantities of the substance concerned in the case of almost all the components in the batch without isomaltulose, the differences are only slight and, moreover, they are probably due to the slightly lower quantity, in absolute terms, of substrate utilised. Moreover, it was found, that *Saccharomyces cerevisiae* MJJ 25 formed higher concentrations of the substance concerned, in some cases, in the isomaltulose-containing solution. By way of the data illustrated here, it cannot be said unequivocally that the presence of isomaltulose influences the aroma profile formed.

Figure 7b shows the content of aroma components after fermentation of real worts by *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* MJJ 2.

Again, no clear-cut influence of the addition of isomaltulose on the formation of the aroma components can be seen. Although, in most cases, less of the substances considered is formed in the worts to which isomaltulose had been added, this is also due to the lower quantity of substrate utilised overall. Worth noting is the fact that *Schizosaccharomyces pombe* again forms slightly more acetaldehyde in the presence of isomaltulose.

In the worts without isomaltulose, less diacetyl and pentanedione is formed. The differences in concentration can be explained not only by the lower conversion of substrate: in the isomaltulose-containing worts, approximately one quarter of the extract was replaced by isomaltulose. However, the contents measured in the worts with isomaltulose are only 50 % or less, compared with the untreated

worts. The presence of isomaltulose in the worts blocks those metabolic pathways which are connected with the formation of the substances of diacetyl and pentanedione. Exceptions in this respect are the worts which are fermented with *Schizosaccharomyces pombe*. This yeast formed more diacetyl and also more pentanedione in the wort with isomaltulose.

The presence of isomaltulose has no influence worth mentioning on the development of substances from the group of the esters and higher aliphatic alcohols (and acetaldehyde). An exception in this respect is the yeast *Schizosaccharomyces pombe* which obviously forms more acetaldehyde if isomaltulose is also present as substrate, instead of maltose. This yeast is also the exception for the formation of vicinal diketones. In the case of the yeasts typical for breweries, the formation of these substances is obviously slowed by the presence of isomaltulose.

#### 7.3.4 Taste assessment

In addition to the chemical analyses, the beers were subjected to a taste assessment on completion of the main and secondary fermentation. On a scale of 1 to 5, the following parameters were assessed: impression of sweetness, impression of bitterness, hop aroma, maltiness, fruitiness, liveliness, palatfulness and overall impression.

The results of the tasting assessment are as follows.

Figure 8a shows the results of the taste assessment of the beers made from the real worts fermented with *Saccharomyces carlsbergensis* MJJ 11 (10 tasters): the aroma profiles resulting from the tasting scheme matched in an almost identical manner after fermentation by *Saccharomyces carlsbergensis* MJJ 11, irrespective of whether isomaltulose was contained in the wort or not. Only with respect to the overall impression was the isomaltulose beer assessed as being better. A frequently mentioned reason was a "more rounded" taste impression although the individual parameters were assessed identically.

Figure 8b shows the results of the taste assessment of the beers made from the real worts; fermented with *Saccharomyces cerevisiae* MJJ 25 (10 tasters): After

fermentation with *Saccharomyces cerevisiae* MJJ 25, too, the aroma profiles matched in an almost identical manner. Again, the isomaltulose-containing beer was assessed as being slightly better in the overall assessment, although the individual parameters were assessed identically. After fermentation with *Saccharomyces cerevisiae* MJJ 25, however, both the bitterness impression and the fruitiness of the beers was perceived more strongly.

Figure 8c shows the results of the taste assessment of the beers made from the real worts; fermented with *Saccharomyces cerevisiae* MJJ 2 (10 tasters): Following fermentation with *Saccharomyces cerevisiae* MJJ 2, the beers were again assessed fairly similarly. The beer without isomaltulose was perceived as less sweet, instead the impression of bitterness became more dominant though, on the other hand, it was obviously somewhat compensated by the presence of isomaltulose. Both beers were assessed identically for overall quality.

Figure 8d shows the results of the taste assessment of the beers made from the real worts; fermented with *Schizosaccharomyces pombe* (10 tasters): Following fermentation by *Schizosaccharomyces pombe*, the beers clearly differed substantially. The isomaltulose-containing beer was perceived as sweeter, though obviously this sweetness was perceived as being malty. Although the intensity of bitterness was perceived equally strongly, this bitterness was perceived as slightly more hop-aromatic in the case of beers without isomaltulose, an impression which was obviously compensated by the isomaltulose contained therein. However, when assessing the overall quality, the isomaltulose-containing beer was again assessed as being slightly better.

The isomaltulose-containing beers were perceived in the majority of cases not as substantially different from the reference beers. Regarding the overall quality, the addition may cause the impression of the beer to appear somewhat "rounder" by compensating for negative influences on the taste such as e.g. strong bitterness.

Example 8: Isomaltulose utilisation of bacteria and influence of beer-inherent selective factors on the isomaltulose utilisation of yeasts.

### 8.1 Production of the medium

A model solution containing 5 % isomaltulose and 6.7 g/l YNB (yeast nitrogen base) was produced in a sterile manner. Incubation took place at 26 °C on a 10 ml scale in test tubes with Durham tubes.

The selective factors were adjusted as follows:

- manipulation of the pH value by means of the addition of phosphoric acid
- content of bitter compounds by means of the addition of isohumulones,
- alcohol content by means of the addition of 96 % non-denatured ethanol and
- exclusion of oxygen by incubation in anaerobic pots.

The isomaltulose utilisation was measured by checking the gas development (visually), measurement by means of the DSN method (photometrically) and analysis of the sugar spectrum (HPLC).

### 8.2 Micro-organisms investigated and analyses

The isomaltulose utilisation of the following yeasts was examined:

- *Saccharomyces carlsbergensis* MJJ 11 (brewer's yeast);
- *Saccharomyces cerevisiae* MJJ 2 (brewer's yeast, good utiliser of isomaltulose);
- *Schizosaccharomyces pombe* (good utiliser of isomaltulose) and
- *Saccharomyces diastaticus* (substance harmful to beer, capable of superfermentation).

In addition, the isomaltulose utilisation of known bacteria harmful to beer was examined. For this purpose, "ideal conditions", anaerobic, 28 °C, 21 days incubation, were selected:

- *Pediococcus damnosus* (DSM: 20331),
- *Megasphaera cerevisiae* (wild strain),
- *Pectinatus frisingensis* (DSM: 20465) and

- *Lactobacillus brevis* (DSM: 20054).

The term "wild strain" means that it is a strain isolated from contaminated beer which does not have a DSM number (DSM: Deutsche Sammlung von Mikroorganismen – German collection of micro-organisms).

In addition, further Lactobacilli were examined because of the importance of Lactobacilli as substances harmful to beer and as probiotic cultures in the food industry:

- *L. fructivorans* (DSM: 20203),
- *L. fructivorans* (wild strain),
- *L. corniformis* (DSM: 20001),
- *L. lindneri* (DSM: 20690),
- *L. lindneri* (DSM: 20961),
- *L. casei* (DSM: 2001),
- *L. curvatus* (wild strain),
- *L. brevis* (DSM: 6235),
- *L. brevis* (Wild strain),
- *L. acidophilus* (DSM: 20242),
- *L. amylovorus* (DSM: 20552),
- *L. delbrückii* (DSM: 20047),
- *L. fermentum* (DSM: 20049),
- *L. gasseri* (DSM:20077),
- *L. johnsonii* (DSM: 20553),
- *L. plantarum* (DSM: 12028),
- *L. reuteri* (DSM: 20015),
- *L. rhamnosus* (DSM: 20023) and
- *L. salivarius* (DSM: 20492).

Since it is known from the literature about Lactobacilli, that these bacteria species are not capable of synthesising all amino acids, experiments were carried out in parallel with the untreated medium, in which the medium additionally contained 2 % peptone. Because of the classification, known from

the literature, of *Lactobacilli* into hop-tolerant and hop-intolerant ones, a third test series was carried out in which the medium contained 20 mg/l of isohumulones.

The concentration of isomaltulose was determined by HPLC as being 42,3 g/l. This value was compared with the residual contents after incubation given below, as initial value. The model medium was incubated unchanged and with an addition of beer-typical selective factors.

### 8.3 Results

#### 8.3.1 Yeasts

After an incubation period of 14 days, the results illustrated in Figure 9 were obtained for *Saccharomyces carlsbergensis* MJJ 11.

Figure 9a shows isomaltulose contents of the non-incubated model solution and after an incubation period of 14 days (anaerobic, 26 °C) with *Saccharomyces carlsbergensis* MJJ 11 (measured by means of HPLC).

It can be seen that the values measured correspond to the initial value with an accuracy of 5 %. This means that no metabolism of the isomaltulose has taken place. The lowest value was measured in the solution without selective factors, although it is not possible to speak of a decrease by metabolism neither in this case. On the basis of this test series it can be said that *Saccharomyces carlsbergensis* MJJ 11 was unable to ferment isomaltulose independently of selective influences present, in the period considered.

In the following illustration, the corresponding test series with the yeast *Saccharomyces cerevisiae* MJJ 2 is illustrated. This yeast is known from existing series of experiments as capable of utilising isomaltulose.

Figure 9b shows isomaltulose contents of the non-incubated model solution and after an incubation period of 14 days (anaerobic, 26 °C) with *Saccharomyces cerevisiae* MJJ 2 (measured by means of HPLC).

After 14 days incubation, a decrease in the batch without selective factors was measured. However, in this case, too, the decrease was only slight and a similar decrease was not measured in any batch that had been modified.

Figure 9c shows isomaltulose contents of the non-incubated model solution and after an incubation period of 14 days (aerobic 26 °C) with *Saccharomyces cerevisiae* MJJ 2 (measured by means of HPLC).

As expected, *Saccharomyces cerevisiae* MJJ 2 exhibited clearly a better ability of utilising isomaltulose under aerobic conditions. In the non-modified batch, approximately 75 % of isomaltulose had been metabolised after 14 days. In the aerobic atmosphere, moreover, a slight degradation of isomaltulose was determined even at a pH value of 4; however, no isomaltulose utilisation took place with a further decrease in the pH value.

Even the absence of oxygen in the case of this yeast made the utilisation of isomaltulose as substrate much more difficult. The presence of hop bitter compounds and alcohol as well as a decrease in the pH value to below a value of 4 causes the metabolisation of isomaltulose to stop completely.

The same experiment was carried out with *Schizosaccharomyces pombe*. Figure 9d shows the sugar contents of the non-incubated model solution and after an incubation period of 14 days (anaerobic, 26 °C) with *Saccharomyces pombe* (measured by means of HPLC).

It can be clearly recognised that this yeast is under all experimental conditions capable of utilising the isomaltulose offered as substrate. Only in the batch with the lowest adjusted pH value is a measurable residual concentration of isomaltulose still present such that, on further reducing the pH value, the isomaltulose utilisation can possibly be prevented; however, pH values below 3 can be found only in very few beverages and even there they are not far below this value. Selective factors typical for beer, even in combination, such as the batch with 5 % of alcohol and existing hop bitter compounds, are not capable of preventing isomaltulose utilisation.

It was found that *Schizosaccharomyces pombe* is capable of utilising isomaltulose effectively. The values measured for the individual sugars of glucose and fructose suggest that this yeast splits isomaltulose in an extracellular manner before the simple sugars are then assimilated.

A different picture emerged after carrying out the test series with the yeast *Saccharomyces diastaticus*, which is known as a substance harmful to beverages. Figure 9e shows the isomaltulose contents of the non-incubated model solution and after an incubation period of 14 days (anaerobic, 26 °C) with *Saccharomyces diastaticus* (measured by means of HPLC).

In none of the batches examined has any decrease in the isomaltulose concentration been detected. Again, the values remain constant within a range of variation of 5 %, such that the conclusion can be drawn that *Saccharomyces diastaticus* is not capable of attenuating isomaltulose.

#### *8.3.2 Bacteria*

In Figure 10, the results of the incubation tests with four known bacteria harmful to beer are illustrated.

Figure 10a shows the isomaltulose contents of the non-incubated model solution and after an incubation period of 21 days (anaerobic, 28 °C) with selected bacteria harmful to beer (measured by means of HPLC).

In none of the batches has any isomaltulose utilisation been detected within the range of accuracy of measurement. None of the bacteria examined is capable of growing on the basis of isomaltulose.

In view of the significance of the group of *Lactobacilli* not only as a substance harmful to beer but also as an organism in the intestinal and dental flora of mammals, and in view of their suitability for use as probiotic cultures in the food industry, a group of various *Lactobacilli* was examined additionally for its ability to utilise isomaltulose.

Figure 10b shows isomaltulose contents after an incubation period of 21 days (anaerobic, 28 °C) with different Lactobacilli (measured by means of DNS assay).

After the long incubation period of three weeks, the variations in the isomaltulose concentrations are within 5 %. The lowest values were measured for the bacteria *L. lindnerii* 20961, *L. brevis* 6235 and *L. rhamnosus* 20023. The values are within a range of variation of 5 % and, moreover, no cell mass growth was observed visually. It is thus not possible to say on the basis of these measurements that the organisms examined are capable of utilising isomaltulose.

Since it is known from the literature that Lactobacilli are not capable of synthesising all amino acids, a further test series was carried out in which 2 % peptone was added to the model solution. In this way, the possibility was to be excluded that a possible growth cannot be detected only because of absent nitrogen sources.

Figure 10c shows isomaltulose contents after an incubation period of 21 days (anaerobic, 28 °C) with various Lactobacilli, with an additional addition of peptone (measured by means of DNS assay).

Again, no decrease by more than 5 %, compared with initial value, was measured in any of the measurements. However, it is striking that several values approach this limit value fairly closely. In addition, it deserves to be mentioned that in the case of the organisms which had the lowest values in the series of measurements without peptone, fairly low values were again measured.

For organisms such as *Lactobacillus lindnerii* 20961, it is not possible to exclude the possibility with absolute certainty that utilisation of isomaltulose is possible after a corresponding adaptation. However, the possibility should be taken into consideration that an incubation was carried out in this case for three weeks under almost ideal conditions but the isomaltulose concentrations nevertheless decreased only slightly. Consequently, and taking possible inaccuracies of measurement into consideration (some values are higher than

the initial concentration), utilisation cannot be considered as having been proven on the basis of the measurements illustrated here.

Correspondingly, the test series in which hop bitter compounds were added to the solution as inhibitors, showed that in this case, too, the measured values were not lower than 95 % of the initial value, after incubation. Thus, again no utilisation of the isomaltulose was proven in the period considered.

## **Claims**

1. A process for the production of microbiologically stabilised beer or mixed beer beverage from brewing liquor, hops and a source of carbohydrate containing the steps of:

- a) mixing of brewing liquor, hops and the source of carbohydrate to form a wort,
- b) boiling the wort and
- c) microbially fermenting the wort,

characterised in that the source of carbohydrate contains isomaltulose or an isomaltulose-containing mixture as germ-stabilising agent.

2. The process according to claim 1, wherein the source of carbohydrate contains malted grain and/or raw grain.

3. The process according to any one of the preceding claims, wherein isomaltulose or the isomaltulose-containing mixture is contained in the source of carbohydrate in a ratio of the other components of the source of carbohydrate to isomaltulose of 4:1 to 2:1.

4. The process according to any one of the preceding claims, wherein the isomaltulose-containing mixture or the isomaltulose of the source of carbohydrate is added as a syrup, in solution or as a crystalline solid.

5. Microbiologically stabilised beer producible according to the process of any one of claims 1 to 4.

6. Mixed beer beverage containing:

- a) Microbiologically stabilised beer producible or produced according to the process of any one of claims 1 to 4 and

b) at least one further component selected from: extracts of herbs, aroma compounds, caffeine, colorants, amino acids, culinary acids, fruit components such as fruit juice, fruit pulp or fruit extracts, sugars, sugar substitutes such as sugar alcohols, intensive sweeteners, water and distilled spirits (ethanol).

7. Mixed beer beverage according to claim 6 containing isomaltulose as sweetening agent.

8. Mixed beer beverage according to claim 7 containing isomaltulose as only sweetening agent.

9. Mixed beer beverage according to claim 7 containing isomaltulose as only body-providing sweetening agent.

10. Use of isomaltulose as germ-stabilising agent for the low germ production of beer or mixed beer beverage.

11. Use of isomaltulose for the production of microbiologically stabilised beer or mixed beer beverage.