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- (73) Patenthaver: **CARLSBERG BREWERIES A/S, J.C. Jacobsens Gade 1, 1799 København V, Danmark**
- (72) Opfinder: **Donaldson, Iain, Rued Langgaards Vej 9, 2.th, 2300 København S, Danmark**
Gojkovic, Zoran, Carlsberg Breweries A/S, Ny Carlsberg Vej 100, 1799 København V, Danmark
Vaag, Pia, Carlsberg Breweries A/S, Ny Carlsberg Vej 100, 1799 København V, Danmark
- (74) Fuldmægtig i Danmark: **HØIBERG P/S, Adelgade 12, 1304 København K, Danmark**
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DESCRIPTION

Technical field

[0001] The present invention relates to the field of beverages.

Background

[0002] The World Health Organization (WHO) predicts that overweight and obesity may soon be the most significant cause of poor health. Food and beverages supporting a healthy life style are therefore in high demand. Such products should preferably be low in calories, but high in other beneficial nutrients.

[0003] EP 0748 168 relates to an alcohol free refreshing drink, which is prepared by fermenting glucose to gluconic acid using a microorganism. However, after the bacterial fermentation, the resulting liquid contains a high level of gluconic acid and gluconate. In order to reduce this, the beverage has to be diluted, thus reducing the level of other micro nutrients.

[0004] US20120114791 relates to methods for production of alcoholic beverages with reduced content of alcohol. The methods comprise treatment of an unfermented beverage starting solution with glucose oxidase and glucose isomerase, which may lead to a sugar reduction of around 19%. The methods may contain an optional step of removing at least a portion of the gluconic acid generated e.g. by means of neutralization by addition of a substance forming a sparingly soluble salt of gluconic acid, preferably calcium carbonate.

[0005] GB1037725 discloses an apparatus and a process for modifying the ion content of liquid foods while adjusting and controlling the pH of the resulting product. In particular, the document discloses a process for treatment of tea concentrate to produce a less expensive instant tea.

Summary

[0006] The present disclosure provides methods for preparing beverages low in calories and high in beneficial micro-nutrients. In particular, the disclosure provides methods for removal of organic acids during beverage production. As described above, reduction of glucose level may lead to production of acids, e.g. gluconic acid. The present inventors have found that the prior art methods are restricted, because the acid production ultimately inhibits the glucose removal, and thus only relatively low levels of glucose can be removed and/or high levels of acids are produced. Interestingly, the present disclosure provides methods, wherein the generated organic acids continuously are removed, preferably simultaneously with the generation of the

organic acids. Thus, in the methods of the disclosure, the removal of sugar is not impaired by accumulating high levels of organic acids, because the organic acids may be removed continuously.

[0007] Interestingly, the disclosure demonstrates that such beverages are palatable providing a good taste. If the acidity is kept at a low level, then the sugars can be kept at a low level, allowing for a beverage with low calorie content.

[0008] The disclosure provides methods for reducing the level of organic acid, while at the same time retaining the level of one or more micro-nutrients. Interestingly, the methods described herein leads to palatable beverages, e.g. beverages with a good flavour and taste. The important aroma compounds are retained in the beverages. Another interesting feature of the beverages prepared according to the methods of the disclosure is that even in absence of adding non-natural ingredients, the beverages are palatable and contain a good level of micronutrients.

[0009] The invention provides a method of preparing a beverage, wherein the method comprises the steps of a) providing a starting liquid comprising at least one micro-nutrient and at least one organic acid, wherein the micro-nutrient is selected from the group consisting of minerals, vitamins, salts and anti-oxidants; and d) removing at least some of said organic acid from said liquid, wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack, said membrane stack containing i) at least one cell consisting of: a. two anion exchange membranes defining a chamber for the starting liquid; and b. two further chambers for a dialysis liquid, wherein said two further chambers are positioned adjacent to the chamber for the starting liquid on opposing sides and wherein said two further chamber may be connected to ii) a set of end membranes iii) means for applying an electric field over the membrane stack by means of at least two electrodes iv) means for reversing the direction of the electric field within said membrane stack and wherein the removal involves the steps of I. inserting the starting liquid into the chamber for the starting liquid; and II. inserting a dialysis liquid in the two further chambers for the dialysis liquid; and III. applying an electric field over the membrane stack; IV. incubating said starting liquid in said chamber, whereby the direction of electric field is reversed at intervals and e) removing at least part of one cation from the starting liquid or from partly AX-REED treated liquid, thereby obtaining a REED liquid, wherein said cation is removed through a Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack, said membrane stack containing i) at least one cell consisting of: a. two cation exchange membranes defining a chamber for the starting liquid or the partly AX-REED treated liquid; and b. two further chambers for a second dialysis liquid, wherein said two further chambers are positioned adjacent to the chamber for the starting liquid or the partly AX-REED treated liquid on opposing sides and wherein said two further chambers may be connected to v) a set of end membranes vi) means for applying an electric field over the membrane stack by means of at least two electrodes vii) means for reversing the direction of the electric field within said membrane stack and wherein the removal involves the steps of I. inserting the starting liquid or the partly AX-REED treated liquid into the chamber for the starting liquid or the partly AX-REED treated liquid; and II. inserting a second

dialysis liquid in the two further chambers for the second dialysis liquid; and III. applying an electric field over the membrane stack; IV. incubating said starting liquid or said partly AX-REED treated liquid in said chamber, whereby the direction of electric field is reversed at intervals wherein steps d) and e) are performed at least partly simultaneously, and wherein the AX-REED membrane stack and the CX-REED membrane stack are connected to a tank, and the AX-REED receives starting liquid from the tank and said starting liquid is returned to the tank after being treated in the AX-REED stack and the CX-REED receives starting liquid or partly AX-REED treated liquid or AX-REED liquid from the tank and said liquid is returned to the tank after being treated in the CX-REED stack, and wherein at least 65% of the at least one micro-nutrient is retained in the REED liquid.

[0010] The starting liquid may be incubated in the chamber for the starting liquid for a predetermined retention time.

Description of Drawings

[0011]

Figure 1 shows an overview of a REED equipment.

Figure 2 shows consumption of maltose and glucose during two consecutive REED controlled fermentations.

Figure 3 shows the concentration of citric acid, malic acid and ascorbic acid in lemon juice during REED treatment over time. Panel A) shows treatment for 4.5 h, whereas panel B) shows treatment for 3.5 h.

Figure 4 shows an exemplary REED equipment.

Figure 5 shows the preference for beverages with either 22 g/L, 37 g/L or 52 g/L of glucose in a test panel of 75 persons. There is a clear preference for the beverage containing 37 g/L.

Figure 6 shows beer flavour profiles for Beverage A and Beverage B as evaluated by a trained taste panel. Full line: Beverage A (REED-based).
Dotted line: Beverage B (based on diluted wort).

Figure 7 shows the pH profile during trial 59 (panel A)) and during trial 60 (panel B)).

Detailed description

[0012] The invention is defined by the appended claims. Any embodiment not falling under the scope of the appended claims does not form part of the invention.

Method of producing a beverage

[0013] The present disclosure relates to methods for producing a beverage or a drinks base, wherein said drinks base may be processed into a beverage by addition of one or more flavour compounds.

[0014] In particular, the methods of the disclosure are useful for preparing beverages with a ratio of sugar to organic acid of in the range of 60:1 to 1:2, which the present inventors have found to be particularly palatable. Said ratio of sugar to organic acid may be any of the ratios described herein below in the section "Ratio of sugar to organic acid".

[0015] The methods of the disclosure are also useful for preparing beverages with reduced acidity.

[0016] The methods of the disclosure in general comprise the steps of:

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose; and
3. c) optionally incubating said liquid with
 1. (i) one or more glucose fermenting microorganisms and/or
 2. (ii) an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to an organic acid; and
4. d) removing at least 10% of one or more acidic ions from said liquid while retaining said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid, wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0017] Step d) may in particular removing at least 10% of one or more acidic ions from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid.

wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack

[0018] The AX-REED liquid may be the final beverage, however the AX-REED liquid may also be further processed to obtain the final beverage. Thus in one embodiment of the disclosure, the methods comprises performing steps a), b), c) and d), wherein the beverage is the obtained AX-REED liquid.

[0019] The starting liquid of step a) may be any liquid useful as a starting liquid for preparation of a beverage. In particular, it may be any of the starting liquids described herein below in the

sections "Method of producing fermented beverage" and "Method of producing a beverage without bacterial fermentation" and "Method of producing a beverage with enzymatic conversion of sugar".

[0020] The micro-nutrient of step a) may be any of the micro-nutrients described herein below in the section "Micro-nutrient".

[0021] The sugar of the starting liquid may be any of the sugars described in the section "Sugar".

[0022] Step b) of the method is an optional step, which may be performed in order to convert one or more sugars to glucose. Whether or not this step is performed will be dependent on whether the starting liquid contains other sugars than glucose and on whether it is desirable to convert one or more of said other sugars to glucose.

[0023] In some embodiments of the disclosure, the method mainly removes organic acids from the starting liquid to produce a beverage or drink base with less acidity. In these embodiments, if the starting liquid comprises a sugar level and composition, which is also desirable in the final beverage or drink base, then step b) will in general be excluded.

[0024] In other embodiments of the disclosure the method comprises a fermentation step (step c (i))). In these embodiments, it is preferable that if the starting liquid only comprises low levels of glucose or if the starting liquid comprises high levels of other sugars than glucose, then step b) is performed. Step b) may be formed in any of the ways described herein below in the section "Converting sugar to glucose".

[0025] Step c) is also an optional step. In step c (i) the glucose level is reduced by fermenting glucose preferably to obtain an organic acid. In general step c) is performed in embodiments of the disclosure where the starting liquid comprises a sugar level, which is higher than desirable. Thus, step c), such as step c (i) is in particular part of the method in embodiments of the disclosure where the starting liquid comprises more than 10%, for example more than 9%, such as more than 8%, for example more than 7% sugar. Said percentages are given as w/w. The sugar may be any of the sugars described herein below in the section "Sugar". Step c (i) may be performed in any of the ways described herein below in the section "Incubation with glucose fermenting microorganism".

[0026] In other embodiments of the disclosure the method comprises a step c, which comprises incubation with an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to an organic acid (step c (ii)). In these embodiments, it is preferable that if the starting liquid only comprises low levels of glucose or if the starting liquid comprises high levels of other sugars than glucose, then step b) is performed. Step b) may be formed in any of the ways described herein below in the section "Converting sugar to glucose".

[0027] Step c (ii) comprising incubation with an enzyme or a mixture of enzymes capable of

catalysing conversion of glucose to an organic acid is also an optional step. Step c (ii) reduces the glucose level by enzymatically degrading glucose to obtain organic acid(s). In general step c) is performed in embodiments of the disclosure where the starting liquid comprises a sugar level, which is higher than desirable. Thus, step c), such as step c (ii) is in particular part of the method in embodiments of the disclosure where the starting liquid comprises more than 10%, for example more than 9%, such as more than 8%, for example more than 7% sugar. Said percentages are given as w/w. The sugar may be any of the sugars described herein below in the section "Sugar". Step c (ii) may be performed in any of the ways described herein below in the section "Incubation with glucose degrading enzyme".

[0028] Removal of one or more acidic ions from the liquid is performed using AX-REED membrane stack. As used herein the term "removing an organic acid" refers to removing the acidic ion of said organic acid. The AX-REED membrane stack may be any of the AX-REED membrane stacks described herein below in the section "AX-REED" and the removal may be performed in any of the ways described herein below in the section "AX-REED". Removal of said acidic ions is preferably performed in a manner, wherein at least one micro-nutrient is retained in the liquid. Said micro-nutrient may be any of the micro-nutrients described herein below in the section "Micro-nutrient". The term "retaining said at least one micro-nutrient" as used herein means that the concentration of said at least one micro-nutrient has not decreased by more than 30%, preferably not by more than 20%, such as not more than 10%, for example the concentration of said micro-nutrient has not decreased by more than 5% during performance of step d). Even more preferably "retaining said at least one micro-nutrient" means that the concentration of said micro-nutrient is the same or higher before and after performance of step d).

[0029] Step d) in general involves removal of acidic ions by the following steps:

1. i) inserting the starting liquid into the chamber for the starting liquid in the AX-REED membrane stack; and
2. ii) inserting a dialysis liquid in the two further chambers for the dialysis liquid in the AX-REED membrane stack; and
3. iii) applying an electric field over the membrane stack;
4. iv) incubating said starting liquid in said chamber, whereby the direction of electric field is reversed at intervals.

[0030] Said incubation of the starting liquid in said chamber may be performed for a predetermined retention time. The predetermined retention time may be selected according to the specific method. In general the methods described in the section "Method of producing a beverage without bacterial fermentation" requires shorter retentions times. Whereas the methods described in the sections "Methods of producing a fermented beverage" and "Methods of producing a beverage with enzymatic conversion of sugar" in general requires longer retention times. The retention time may be selected to obtain a desired pH. In particular the retention time may be selected to obtain a desirable contact time, which may be any of the

contact times described herein below in the section "Contact time".

[0031] In addition to steps a) to d) outlined above, the methods of the disclosure may also comprise a step e), wherein step e) comprises removing at least part of one cation from the liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack. The CX-REED membrane stack may be any of the CX-REED membrane stacks described herein below in the section "CX-REED" and step e) may be performed in any of the ways described in the section "CX-REED". Thus, in one embodiment the disclosure relates to a method for preparing a beverage with reduced acidity, said method comprising the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose; and
3. c) optionally incubating said liquid with one or more glucose fermenting microorganisms; and
4. d) removing at least 10% of one or more acidic ions from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtain an AX-REED liquid
wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
5. e) removing at least part of one cation from the AX-REED liquid, while retaining said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0032] Step e) may in particular involve removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid,
wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0033] Step e) in general involve removal of cations by the following steps:

1. i) inserting the starting liquid, the partly AX-REED treated liquid or the AX-REED liquid into the chamber for the AX-REED liquid; and
2. ii) inserting a second dialysis liquid in the two further chambers for the second dialysis liquid; and
3. iii) applying an electric field over the membrane stack;
4. iv) incubating said liquid in said chamber, whereby the direction of electric field is reversed at intervals.

[0034] Said incubation of the starting liquid in said chamber may be performed for a predetermined retention time. The predetermined retention time may be selected according to the specific method. The retention time may be selected to obtain a desired conductivity. In particular the retention time may be selected to obtain a desirable contact time, which may be any of the contact times described herein below in the section "Contact time".

[0035] The CX-REED liquid may be the final beverage. However, the CX-REED liquid may also be further processed to obtain the final beverage. For example the CX-REED liquid will be the final beverage or it will be a drink base, which will be a final beverage after addition of one or more additional compounds as described herein below for step f). Thus, in one embodiment the disclosure provides methods of preparing a beverages, wherein the methods comprise performing steps a), b), c), d) an e) as outlined above, wherein the beverage is the CX-REED liquid.

[0036] As described herein elsewhere, then steps d) and e) may be performed simultaneously or partly simultaneously. In these embodiments the resulting liquid may also be referred to as "REED liquid".

[0037] Thus, in one embodiment the disclosure relates to a method for preparing a beverage with reduced acidity, said method comprising the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose; and
3. c) optionally incubating said liquid with one or more glucose fermenting microorganisms; and
4. d) removing at least 10% of one or more acidic ions from said liquid, wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
5. e) at least partly simultaneously removing at least part of one cation from the starting liquid or the partly AX-REED treated liquid, thereby obtaining a REED liquid, wherein said REED liquid retains at least 65% of said at least one micro-nutrient, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0038] The REED liquid may be the final beverage. However, the REED liquid may also be further processed to obtain the final beverage. For example the REED liquid will be the final beverage or it will be a drink base, which will be a final beverage after addition of one or more additional compounds as described herein below for step f). The REED liquid may also be treated with one or both of steps g) and h) described herein below. In addition to the aforementioned steps the method may further comprise a step f), wherein step f) comprises

adding one or more additional compounds to the starting liquid and/or to the liquid during the method and/or to the beverage. Said additional compounds may be any compound desirable to add to a beverage, for example the additional compounds may be one or more selected from the group consisting of flavour compounds and preservatives. The flavour compound may for example be any of the flavour compounds described herein below in the section "Flavour compound".

[0039] Thus, in one aspect the disclosure relates to a method for preparing a beverage, said method comprising the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose; and
3. c) optionally incubating said liquid with one or more glucose fermenting microorganisms; and
4. d) removing at least 10% of one or more acidic ions from said liquid while retaining said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
5. e) removing at least part of one cation from the AX-REED liquid, while retaining said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack; and
6. f) adding one or more additional compounds, preferably one or more additional compound selected from the group consisting of flavour compounds and preservatives, thereby obtaining a beverage.

Method of producing fermented beverage

[0040] As outlined in the above section step c) of the method of the disclosure is an optional step. However, in one preferred embodiment of the disclosure the methods comprise performing step c). In particular the methods of the disclosure may preferably comprise performing step c (i). Thus, in one embodiment the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

- a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
- c) incubating said liquid with
 1. (i) one or more microorganisms capable of fermenting said sugar to produce an organic acid; and
- d) removing at least 10% of one or more acidic ions from said liquid while retaining at least

65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid,

[0041] The microorganism capable of fermenting said sugar to produce an organic acid may be any useful microorganism, with these characteristics. In particular, it is preferred that the microorganism is a glucose fermenting microorganism. Thus, the disclosure provides methods for producing a beverage comprising the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
 2. b) if said sugar is not glucose converting at least some of said sugar to glucose; and
 3. c) incubating said liquid with one or more glucose fermenting microorganisms capable of fermenting glucose to an organic acid; and
 4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid
- wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0042] Said AX-REED liquid may be the final beverage or it may be further processed to obtain the final beverage as described below.

[0043] This method may furthermore comprise step e) and thus in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
 2. b) if said sugar is not glucose converting at least some of said sugar to glucose; and
 3. c) incubating said liquid with one or more glucose fermenting microorganisms capable of fermenting glucose to an organic acid; and
 4. d) removing at least 10% of said organic acid from said liquid while retaining said at least 65% of said one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid wherein said organic is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
 5. e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid,
- wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0044] The CX-REED liquid may be the final beverage or it may be further processed to obtain

the final beverage. It is preferred that the CX-REED liquid is the final beverage or that the final beverage is obtained by adding one or more additional compounds to the CX-REED liquid.

[0045] Step d) and e) may be performed at least partly simultaneously and thus in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose; and
3. c) incubating said liquid with one or more glucose fermenting microorganisms capable of fermenting glucose to an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid,
wherein said organic is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
5. e) at least partly simultaneously removing at least part of one cation from the starting liquid or the partly AX-REED treated liquid, thereby obtaining a REED liquid, wherein said REED liquid retains at least 65% of said at least one micro-nutrient,
wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0046] The REED liquid may be the final beverage or it may be further processed to obtain the final beverage. It is preferred that the REED liquid is the final beverage or that the final beverage is obtained by adding one or more additional compounds to the REED liquid.

[0047] Thus, the method may furthermore comprise a step f) of adding one or more additional compounds to the starting liquid, the AX-REED liquid, the CX-REED liquid or the REED liquid. In embodiments of the disclosure not containing step e), then step f) preferably comprises adding one or more additional compounds to the AX-REED liquid. In one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose; and
3. c) incubating said liquid with one or more glucose fermenting microorganisms capable of fermenting glucose to an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
5. e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid,

wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack; and

6. f) adding one or more additional compounds to the CX-REED liquid, preferably one or more additional compound selected from the group consisting of flavour compounds and preservatives, thereby obtaining the beverage.

[0048] The method may also comprise a step g) of adding one or more additional liquids to the AX-REED liquid, the CX-REED liquid or the REED liquid in order to obtain the final beverage. In particular said additional liquids may be beverages, so that the final beverage is a mixture between the CX-REED liquid and an additional beverage or a mixture between the REED liquid and an additional beverage. Thus, in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose; and
3. c) incubating said liquid with one or more glucose fermenting microorganisms capable of fermenting glucose to an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
5. e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid,
wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack; and
6. f) optionally adding one or more additional compounds to the CX-REED liquid, preferably one or more additional compound selected from the group consisting of flavour compounds and preservatives; and
7. g) providing an additional liquid, for example a beverage and mixing said CX-REED liquid with said additional liquid, thereby obtaining the beverage.

[0049] Said additional liquid may be any liquid, which is desirable to mix with the CX-REED liquid. In particular the additional liquid may be a beverage. In one embodiment of the disclosure said additional liquid is an alcoholic beverage, such as a fermented beverage, such as fermented wort or fruit juice. For example the additional liquid may be selected from the group consisting of beer, wine and cider. The additional liquid may also be a fermented fruit juice obtained by mixing fruit juice with sugar followed by fermentation with yeast to yield a liquid with high alcohol content. Herein such liquids are also referred to as fermented high sugar fruit juice. In this regard the term "high sugar" thus refers to that additional sugar is

added to the fruit juice before fermentation. For example the additional beverage may be fermented fruit juice with added sugar containing at least 10%, such as at least 12% alcohol. Said fermented fruit juice, may for example be fermented apple juice, and may then be referred to as fermented high sugar apple juice.

[0050] The methods of the disclosure may be useful for preparing low alcohol beverage by mixing a conventional alcoholic beverage, with a CX-REED liquid obtained using the same base as used for the conventional alcoholic beverage as starting liquid.

[0051] Thus, in one embodiment of the disclosure the final beverage may be a low alcohol beer, such as a beer containing less than 0.5% alcohol, for example a beer containing less than 0.1% alcohol or even an "alcohol free" beer, which is obtained by diluting a conventional beer with CX-REED liquid or REED liquid prepared according to the present disclosure. Frequently, the CX-REED liquid is low in sugar and in general free of alcohol, but still retains other taste attributes of the starting liquid and accordingly, provides the final beverage with all of these taste attributes. Thus, the CX-REED liquid in general retains one or more aroma compounds present in the starting liquid as described herein below in the section "Aroma compounds". In this embodiment the starting liquid for preparing the CX-REED liquid or the REED liquid preferably comprises a cereal extract, more preferably wort.

[0052] Similarly, in another embodiment of the disclosure the final beverage may be a low alcohol cider, such as a cider containing less than 0.5% alcohol, for example a cider containing less than 0.1% alcohol or even an "alcohol free" cider, which is obtained by diluting a conventional cider with CX-REED liquid or REED-liquid prepared according to the present disclosure. In this embodiment the starting liquid for preparing the CX-REED liquid or the REED liquid preferably comprises or consists of pear juice or apple juice, preferably apple juice.

[0053] In yet another embodiment of the disclosure the final beverage may be a low calorie cider, which is obtained by diluting a conventional fermented high sugar apple juice with the CX-REED liquid or the REED liquid. In this embodiment the starting liquid for preparing the CX-REED liquid preferably comprises or consists of pear juice or apple juice, preferably apple juice.

[0054] Furthermore, in this embodiment of the disclosure the CX-REED liquid preferably has a glucose content of at the most 60 g/L, such as at the most 50 g/L, for example at the most 40 g/L. In embodiments of the disclosure where a REED liquid is prepared, then the REED liquid preferably has a glucose content of at the most 60 g/L, such as at the most 50 g/L, for example at the most 40 g/L.

[0055] In another embodiment of the disclosure the final beverage may be a low alcohol wine, such as a wine containing less than 0.5% alcohol, for example a wine containing less than 0.1% alcohol or even an "alcohol free" wine, which is obtained by diluting a conventional wine with CX-REED liquid or REED liquid prepared according to the present disclosure. In this embodiment the starting liquid for preparing the CX-REED liquid or the REED liquid preferably

comprises or consists of a grape juice.

[0056] It is also comprised within the present disclosure that the methods may comprise a step h) of further processing the AX-REED liquid or the CX-REED liquid or the REED liquid in order to obtain the final beverage. Said further processing may for example be a step of incubation of the AX-REED liquid or the CX-REED liquid or the REED liquid with one or more microorganisms, such as yeast. Said methods may comprise above described step g), and thus the methods may comprise incubation of the liquid obtained in step g) with one or more microorganisms, such as yeast. The microorganisms to be employed in step h) may in particular be yeast, such as Brewer's yeast, for example yeast *Saccharomyces cerevisiae* or *Saccharomyces pastorianus*, formerly known as *S. carlsbergensis*. Thus, in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

- a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
- b) if said sugar is not glucose converting at least some of said sugar to glucose; and
- c) incubating said liquid with one or more glucose fermenting microorganisms capable of fermenting glucose to an organic acid; and
- d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
- e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack; and
- f) optionally adding one or more additional compounds to the CX-REED liquid, preferably one or more additional compound selected from the group consisting of flavour compounds and preservatives; and
- h) incubating said AX-REED liquid or said X-REED liquid with one or more microorganisms, such as yeast, optionally followed removal of said microorganism, thereby obtaining the beverage.

[0057] Said step h) may in particular be included in embodiments of the disclosure where the beverage is a beer, such as a low alcohol beer or an alcohol free beer.

[0058] During step c) organic acid is generated and during step d) at least some of said generated organic acid is removed. As described elsewhere steps c) and d) may be performed simultaneously, and thus the organic acid may be continuously generated by said

microorganisms and at least some of the generated organic acid may continuously be removed through said AX-REED membrane stack. While at least 10% of the organic acid is removed it is contained within the disclosure that at least some of the generated organic acid is maintained in the beverage and thus it is comprised within the disclosure, that the beverage may contain more organic acid than the starting liquid. This is in particular the case, when the starting liquid has a high level of sugar and a low level of organic acids. In order to reach a palatable ratio between sugar and organic acid, such as any of the ratios described herein below in the section "Ratio of sugar to organic acid", then it may be preferable that the beverage contains more organic acid than the starting liquid. Thus, preferably the beverage has a ratio of sugar to organic acid as described herein below in the section "Ratio of sugar to organic acid".

[0059] It is preferred that at least 10% of the generated organic acid is removed during step d). Thus for example at least 15%, for example at least 20%, such as at least 25%, such as at least 30% of the organic acid generated in step c) is preferably removed in step d). Said organic acid may be any of the organic acids described herein below in the section "Organic acid".

[0060] In particular it is preferred that at least 10% of generated lactic acid, citric acid, acetic acid and malic acid is removed during step d). More preferably, at least 10% of generated lactic acid, citric acid and acetic acid is removed during step d). Thus, step d) may in a preferred embodiment of the disclosure comprise removal of at least 10% of the lactic acid, for example at least 15% of the lactic acid, for example at least 20% of the lactic acid, such as at least 25% of the lactic acid, such as at least 30% of the lactic acid generated in step c). This is in particular the case in embodiments of the disclosure, where said glucose fermenting microorganism is capable of fermenting glucose to lactic acid.

[0061] Step d) may in another preferred embodiment of the disclosure comprise removal of at least 10% of the citric acid, for example at least 15% of the citric acid, for example at least 20% of the citric acid, such as at least 25% of the citric acid, such as at least 30% of the citric acid generated in step c). This is in particular the case in embodiments of the disclosure, where said glucose fermenting microorganism is capable of fermenting glucose to citric acid.

[0062] Step d) may in another embodiment of the disclosure comprise removal of at least 10% of the malic acid, for example at least 15% of the malic acid, for example at least 20% of the malic acid, such as at least 25% of the malic acid, such as at least 30% of the malic acid generated in step c). This is in particular the case in embodiments of the disclosure, where said glucose fermenting microorganism is capable of fermenting glucose to malic acid.

[0063] Step d) may in another preferred embodiment of the disclosure comprise removal of at least 10% of the acetic acid, for example at least 15% of the acetic acid, for example at least 20% of the acetic acid, such as at least 25% of the acetic acid, such as at least 30% of the acetic acid generated in step c). This is in particular the case in embodiments of the disclosure, where said glucose fermenting microorganism is capable of fermenting glucose to acetic acid.

[0064] In these embodiments of the disclosure the micro-nutrient may for example be any of the micro-nutrients described herein below in the section "Micro-nutrient"; and the sugar may for example be any of the sugars described in the section "Sugar", and step d) may for example be performed in any of the ways described in the section "AX-REED and step e) may for example be performed in any of the ways described in the section "CX-REED". Said ratio of sugar to organic acid may be any of the ratios described herein below in the section "Ratio of sugar to organic acid".

[0065] In particular, in embodiments of the disclosure relating to methods of producing fermented beverages, then it is preferred that said micro-nutrient is selected from the group consisting of minerals, and in particular from the group of minerals described herein below in the section "Micro-nutrient". Within these embodiments of the disclosure it is preferred that steps d) and e) are performed so that at least 65% of at least 2, preferably of at least 3 minerals selected from the group consisting of calcium, magnesium and iron are retained in the liquid. Thus, it is preferred that the final beverage contains at least 65% of the level of at least 2, preferably of at least 3, minerals present in the starting liquid, wherein said minerals are selected from the group consisting of calcium, magnesium and iron present in the starting liquid. Within these embodiments of the disclosure it is also preferred that steps d) and e) are performed so that at least 80% of at least 2, preferably of at least 3 minerals selected from the group consisting of calcium, magnesium and iron are retained in the liquid. Thus, it is preferred that the final beverage contains at least 80% of the level of at least 2, preferably of at least 3 minerals present in the starting liquid, wherein said minerals are selected from the group consisting of calcium, magnesium and iron present in the starting liquid.

[0066] The starting liquid may be any liquid useful for preparing a beverage. It is generally preferred that the starting liquid is a natural product. The term "natural product" as used herein refers to a product obtain from natural sources by extraction in water or by squeezing, wherein no extra chemicals are added. Thus, in one embodiment of the disclosure the starting liquid is an extract, concentrate or a juice of a plant or a plant part, where no extra sugar has been added.

[0067] In embodiments of the disclosure relating to producing a fermented beverage, it is preferred that the starting liquid has a relatively high level of one or more sugars, for example a high level of one or more of the sugars described herein below in the section "Sugar". Thus, the starting liquid may for example comprises more than 10%, for example more than 9%, such as more than 8%, for example more than 7% sugar. Said percentages are given as w/w.

[0068] In one embodiment of the disclosure the starting liquid is a liquid with a high level of maltose. Thus the starting liquid may be a liquid containing more than 40 g/L, such as more than 50 g/L, for example more than 60g/L maltose.

[0069] In particular, the starting liquid may be an extract of one or more cereals. Said cereals may for example be selected from the group consisting of barley, wheat, rye, oat, maize, rice,

sorghum, millet, triticale, buckwheat, fonio and quinoa. More preferably, the cereal is selected from the groups consisting of barley, wheat, rye, oat, maize and rice, more preferably the cereal is barley.

[0070] The extract may be an extract of the cereal per se, however the cereal may also be malted and the extract may be an extract of the malted cereal. As used herein the term "malted", refers to the cereal grains having been subjected to steeping, allowed to germinate and then dried. Said drying may for example be kiln drying.

[0071] Said extract of a cereal or a malted cereal is preferably an aqueous extract.

[0072] Thus, in a preferred embodiment of the disclosure, and in particular in embodiments of the disclosure relating to methods of producing a fermented beverage, then the starting liquid may be a malt extract, such as a barley malt extract. More preferably the starting liquid may be wort. The starting liquid may also be an extract of a mixture of barley malt and other cereals.

[0073] In a particularly preferred embodiment of the disclosure the starting liquid is wort. By the term "wort" as used herein is meant a liquid extract of malt. Wort may also be prepared by incubating an extract of un-malted barley with an enzyme mixture that hydrolyzes the barley components. In addition to said malt or barley-derived extracts, the wort may be prepared from malt and additional components, such as additional starch-containing material partly converted into fermentable sugars. The wort is in general obtained by mashing, optionally followed by sparging.

[0074] The term "Mashing" as used herein refers to the incubation of milled malt in water. Mashing is preferably performed at a specific temperature, and in a specific volume of water. Mashing can occur in the presence of adjuncts, which is understood to comprise any carbohydrate source other than malt, such as, but not limited to, unmalted barley, barley syrups, or maize, or rice - either as whole kernels or processed products like grits, syrups or starch.

[0075] The term "sparging" as used herein refers to a process of extracting residual sugars and other compounds from spent grains after mashing with hot water. Sparging is typically conducted in a lauter tun, a mash filter, or another apparatus to allow separation of the extracted water from spent grains.

[0076] The wort obtained after mashing is generally referred to as "first wort", while the wort obtained after sparging is generally referred to as the "second wort". If not specified, the term wort may be first wort, second wort, or a combination of both.

[0077] Thus, the starting liquid may be wort, such as first wort, second wort or a mixture thereof.

[0078] It is also comprised within the disclosure that the starting liquid may be an extract, a

concentrate or a juice of a plant or a plant part, which has been treated with one or more enzymes. For example said extract, concentrate or juice of a plant or a plant part, may have been treated with one or more enzymes selected from the group consisting of glucosidases, proteases, pectinases and cellulases. In embodiments of the disclosure, where the starting liquid is wort, the said starting liquid may have been prepared employing a step of enzyme treatment with one or more enzymes selected from the group consisting of glucosidases, proteases, pectinases and cellulases, preferably from the group consisting of glucan 1,4- α -glucosidases, proteases, pullulanases, α -amylases, β -amylases, limit dextrinases and β -glucosidases. Treatment with glucan 1,4- α -glucosidases may be considered as step b) of the methods of the disclosure.

[0079] However in one embodiment it is preferred that no proteases are added at any step during the procedure.

[0080] In one embodiment of the disclosure the starting liquid is a fruit juice or extract containing a high level of sugar. In particular, the starting liquid may be a fruit juice or extract, which naturally contains a high level of sugar, such as more than 40 g/L, such as more than 50 g/L, for example more than 60g/L sugar. Said fruit juice may for example be grape juice, pear juice or apple juice. The methods of the disclosure may be used to produce a palatable beverage with reduced sugar content compared to the fruit juice or extract, while retaining one or more valuable micronutrients.

[0081] In certain embodiments of the disclosure the pH of the starting liquid may be adjusted by addition of base or acid, such as potassium hydroxide or lactic acid. This may for example be done in order to start the fermentation at a pH agreeable to the microorganism, such as the glucose fermenting microorganism.

[0082] In embodiments of the disclosure, wherein the starting liquid comprises a malt extract and/or wort, then the starting liquid in general will comprise high levels of maltose, and in these embodiments of the disclosure the methods preferably comprises a step of converting at least some of said maltose to glucose.

[0083] Accordingly, it is also one aspect of the disclosure to provide methods of preparing a beverage, wherein the methods comprise the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and maltose, wherein said starting liquid for example may comprise or even consist of a malt extract and/or wort; and
2. b) converting at least some of said maltose to glucose; and
3. c) incubating said liquid with one or more glucose fermenting microorganisms capable of fermenting glucose to an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid,

wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack as described herein below in the section "AX-REED"

and optionally the method further comprises one of both of the steps e) and f) as outlined above.

[0084] Step b) of converting maltose to glucose may be performed in any of the ways described below in the section "Converting sugar to glucose",

[0085] Step c) of the methods for producing a fermented beverage as described in this section may be performed in any of the ways described herein below in the section "Incubation with glucose fermenting microorganism".

[0086] Step b) and step c) may be performed sequentially, by first performing step b) and then step c). It is however also comprised in the disclosure that step b) and step c) can be performed simultaneously or at least partly simultaneously. For example, when step b) is performed with the aid of an enzyme as described in the section "Converting sugar to glucose" herein below, then the enzyme may be added to the starting liquid together with the glucose fermenting microorganism. When step b) is performed with the aid of a microorganism such as a maltose catabolising microorganism as described in the section "Converting sugar to glucose" herein below, then the starting liquid may be incubated with said microorganism and with the glucose fermenting microorganism simultaneously. In embodiments of the disclosure where step b) is performed with the aid of a microorganism it is preferred that step b) and step c) are performed simultaneously.

[0087] Steps c) and d) may also be performed sequentially, by first performing step c) and then step d). It is however preferred that step c) and step d) are performed simultaneously or at least partly simultaneously. Thus, the liquid may be incubated with one or more glucose fermenting microorganisms capable of fermenting glucose to an organic acid and simultaneously at least some of said organic acid is removed from the liquid. Thus, as the organic acid is produced from the glucose fermenting microorganism, then it is removed from the liquid, ensuring a constant low level of said organic acid in the liquid during the fermentation.

[0088] Accordingly, the steps b), c) and d) may all be performed simultaneously. Alternatively, step b) may be performed first, and then steps c) and d) may be performed simultaneously.

[0089] In general step d) will be performed prior to step e), however it is also contained within the disclosure that steps d) and e) may be performed simultaneously. Preferably however, step d) is performed prior to step e).

[0090] In another very preferred embodiment of the disclosure steps d) and e) are performed simultaneously. Thus, in this embodiment steps b), c), d) and e) may all be performed

simultaneously. Alternatively, steps b) may be performed first and then steps c), d) and e) may be performed simultaneously. Steps d) and e) may in particular be performed simultaneously by using a REED equipment containing at least one AX-REED and at least one CX-REED membrane stack, wherein said AX-REED and said CX-REED membrane stacks are connected in parallel. In yet another very preferred embodiment of the disclosure steps d) and e) are performed partly simultaneously. In this embodiment step d) may for example be performed for a given time period, where after both steps d) and e) are performed simultaneously. Thus, one or more acidic ions may be removed from the liquid through AX-REED for a given time period, where after both acidic ions and at least one cation are removed respectively through AX-REED and CX-REED, wherein the AX-REED and the CX-REED are performed simultaneously. Thus, in this embodiment steps b), c), d) and e) may all be performed at least partly simultaneously. Alternatively, steps b) may be performed first and then steps c), d) and e) may be performed at least partly simultaneously. When steps d) and e) are performed at least partly simultaneously, this is preferably done by using a REED equipment containing at least one AX-REED and at least one CX-REED membrane stack, wherein said AX-REED and said CX-REED membrane stacks are connected in parallel.

In one embodiment of the disclosure it is preferred that no sugar is added to starting liquid and furthermore, it is preferred that no sugar is added at any step during the method. In addition it is preferred that no sugar is added to the final beverage.

[0091] It is comprised within the disclosure that any of the methods may comprise as a last step, the step i) of adding CO₂ in order to obtain a carbonated beverage.

Converting sugar to glucose

[0092] The methods of the disclosure may comprise a step b) of converting at least some sugar, which is not glucose to glucose. This step is an optional step, which may be performed only in embodiments of the disclosure where it is desirable to reduce the level of sugar or in embodiments of the disclosure where it is desirable to reduce the level of a particular sugar, which is not glucose.

[0093] The sugar may for example be selected from the group consisting of fructose, maltose, maltotriose, lactose and sucrose. Thus step b) may comprise one or more of the following:

1. i. Converting fructose to glucose
2. ii. Converting maltose to glucose
3. iii. Converting maltotriose to glucose
4. iv. Converting lactose to glucose
5. v. Converting sucrose to glucose

[0094] In particular step b) may comprise one or more of the following:

1. i. Converting maltose to glucose
2. ii. Converting maltotriose to glucose
3. iii. Converting sucrose to glucose

[0095] Said conversion may be done by any suitable method known to the skilled person. In one embodiment of the disclosure this may be done enzymatically by contacting the starting liquid with an enzyme capable of catalysing conversion the particular sugar in question to glucose. This may also be done employing one or more microorganisms capable of catabolising said sugar to form glucose.

[0096] In preferred embodiments of the disclosure, then step b) comprises converting maltose to glucose. In addition, step b) may comprise converting maltotriose to glucose. This is in particular the case in embodiments of the disclosure where the starting liquid comprises maltose and/or maltotriose, for example in embodiments of the disclosure where the starting liquid comprises a malt extract and/or wort.

[0097] Thus step b) may comprise converting at least some of said maltose to glucose and at least some of said maltotriose to glucose by contacting the starting liquid with one or more enzymes capable of catalysing hydrolysis of maltose to glucose and maltotriose to glucose.

[0098] Thus step b) may comprise converting at least some of said maltose to glucose by contacting the starting liquid with an enzyme capable of catalysing hydrolysis of maltose to glucose.

[0099] Said enzyme may in a preferred embodiment of the disclosure be an enzyme capable of catalysing hydrolysis of terminal (1→4)-linked α -D-glucose residues successively from non-reducing ends of an oligosaccharide resulting in release of β -D-glucose. In particular, the enzyme is preferably capable of catalysing hydrolysis of maltose. The enzyme may in particular be an enzyme classified under EC 3.2.1.3. Thus the enzyme may be a glucan 1,4- α -glucosidase. Said glucan 1,4- α -glucosidase may be of any useful source organism, for example it may be a glucan 1,4- α -glucosidase of microbial origin or of plant origin. In one embodiment of the disclosure the enzyme is a glucan 1,4- α -glucosidases of SEQ ID NO: 1 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. In one embodiment of the disclosure the enzyme is a glucan 1,4- α -glucosidases of SEQ ID NO: 2 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. In one embodiment of the disclosure the enzyme is a glucan 1,4- α -glucosidases of SEQ ID NO: 3 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. A functional homologue of a glucan 1,4- α -glucosidase is a polypeptide capable of catalysing hydrolysis of terminal (1→4)-linked α -D-glucose residues successively from non-reducing ends of an

oligosaccharide with release of β -D-glucose and which have the aforementioned sequence identity with a reference glucan 1,4- α -glucosidase.

[0100] Said enzyme may also be an enzyme capable of catalysing endohydrolysis of (1 $\rightarrow$ 4)- α -D-glucosidic linkages in polysaccharides containing three or more (1 $\rightarrow$ 4)- α -linked D-glucose units. The enzyme may in particular be an enzyme classified under EC 3.2.1.1. Thus the enzyme may be an α -amylase. Said α -amylase may be of any useful source organism, for example it may be a α -amylase of microbial origin or of plant origin. In one embodiment of the disclosure the enzyme is an α -amylase of SEQ ID NO: 4 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. In one embodiment of the disclosure the enzyme is an α -amylase of SEQ ID NO: 5 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. In one embodiment of the disclosure the enzyme is an α -amylase of SEQ ID NO: 6 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. A functional homologue of an α -amylase is a polypeptide capable of catalysing endohydrolysis of (1 $\rightarrow$ 4)- α -D-glucosidic linkages in polysaccharides containing three or more (1 $\rightarrow$ 4)- α -linked D-glucose units and which have the aforementioned sequence identity with a reference α -amylase.

[0101] Said enzyme may also be an enzyme capable of catalysing lysis of (1 $\rightarrow$ 6)- α -D-glucosidic linkages in pullulan, amylopectin and glycogen, and in the α - and β -limit dextrins of amylopectin and glycogen. The enzyme may in particular be an enzyme classified under EC 3.2.1.41. Thus the enzyme may be a pullulanase. Said pullulanase may be of any useful source organism, for example it may be a pullulanase of microbial origin. In one embodiment of the disclosure the enzyme is a pullulanase of SEQ ID NO: 7 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. In one embodiment of the disclosure the enzyme is a pullulanase of SEQ ID NO: 8 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. In one embodiment of the disclosure the enzyme is a pullulanase of SEQ ID NO: 9 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. A functional homologue of a pullulanase is a polypeptide capable of catalysing lysis of (1 $\rightarrow$ 6)- α -D-glucosidic linkages in pullulan, amylopectin and glycogen, and in the α - and β -limit dextrins of amylopectin and glycogen and which have the aforementioned sequence identity with a reference pullulanase.

[0102] The determination of percent sequence identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated

into the BLASTN and BLASTP programs of Altschul, et al. (1990) J. Mol. Biol. 215:403-410.

[0103] In order to characterize the identity, subject sequences are aligned so that the highest order homology (match) is obtained. Based on these general principles the "percent identity" of two amino acid sequences may be determined using the BLASTP algorithm [Tatiana A. Tatusova, Thomas L. Madden: Blast 2 sequences - a new tool for comparing protein and nucleotide sequences; FEMS Microbiol. Lett. 1999 174 247-250], which is available from the National Center for Biotechnology Information (NCBI) web site (<http://www.ncbi.nlm.nih.gov>), and using the default settings suggested here (i.e. Matrix = Blosum62; Open gap = 11; Extension gap = 1; Penalties gap x dropoff = 50; Expect = 10; Word size = 3; Filter on). The BLAST algorithm performs a two-step operation by first aligning two sequences based on the settings and then determining the % sequence identity in a range of overlap between two aligned sequences. In addition to % sequence identity, BLASTP also determines the % sequence similarity based on the settings.

[0104] Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the FASTA sequence alignment software package (Pearson WR, Methods Mol Biol, 2000, 132:185-219). Align calculates sequence identities based on a global alignment. Align0 does not penalise to gaps in the end of the sequences. When utilizing the ALIGN or Align0 program for comparing amino acid sequences, a BLOSUM50 substitution matrix with gap opening/extension penalties of -12/-2 is preferably used.

[0105] Sequence identity according to the present disclosure is determined over the entire length of the reference sequence.

[0106] The starting liquid may be contacted by said enzyme capable of catalysing conversion of a sugar to glucose, for example an enzyme capable of catalysing hydrolysis of maltose to glucose, at any suitable time during the methods. Thus, this step may be performed prior to performed step c). It is also possible that this is done simultaneously with step c). It is furthermore possible that this is done at the same time as preparing the starting liquid. Thus, the starting liquid may be prepared by contacting any suitable liquid with said enzyme and optionally one or more additional compounds.

[0107] In one embodiment of the disclosure the starting liquid is prepared by contacting an extract of a plant or a fruit, e.g. a malt extract with an enzyme capable of catalysing conversion of a sugar to glucose, for example an enzyme capable of catalysing hydrolysis of maltose to glucose as well as with one or more additional enzymes. The enzyme capable of catalysing conversion of a sugar to glucose may in particular be a glucan 1,4- α -glucosidases, whereas the additional enzymes for example may be one or more selected from the group consisting of proteases, pullulanases, α -amylases, β -amylases, β -glucosidases, pectinases and cellulases.

[0108] Step b) of the methods of the disclosure may in one embodiment comprise contacting

the starting liquid with one or more enzymes selected from the group consisting of enzymes classified under EC 3.2.1.3, enzymes classified under EC 3.2.1.1 and enzymes classified under EC 3.2.1.41. In particular, Step b) of the methods of the disclosure may in one embodiment comprise contacting the starting liquid with one or more enzymes selected from the group consisting of glucan 1,4- α -glucosidases, α -amylases and pullulanases, wherein said glucan 1,4- α -glucosidase, α -amylase and pullulanase may be any of the glucan 1,4- α -glucosidases, α -amylases and pullulanases described herein above.

[0109] The enzymes may be provided in any suitable manner for example as recombinant polypeptides or purified from a source organism. The enzyme may also be provided within an enzyme mixture or as a crude extract of the source organism. Source organisms may for example be *Aspergillus* spp. or *Rhizopus* spp. This may in particular be the case for glucan 1,4- α -glucosidases and α -amylases. The source organism may also be *Bacillus* spp. or *Lactobacillus* spp. This may in particular be the case for pullulanase. Suitable enzyme preparations are commercially available for example from Novozymes, Denmark.

[0110] The step b) may comprise converting at least 50%, such as at least 60%, for example at least 70%, such as at least 80%, for example at least 90% of the sugar in the starting liquid, which is not glucose into glucose.

[0111] Thus, step b) may comprise converting at least 50%, such as at least 60%, for example at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 98% of the maltose in the starting liquid to glucose, In particular, when the starting liquid is wort, step b) may comprise converting at least 50%, such as at least 60%, for example at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 98% of the maltose in said wort to glucose,

[0112] Step b) may comprise converting at least 50%, such as at least 60%, for example at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 98% of the maltotriose in the starting liquid to glucose,

[0113] Step b) may comprise converting at least 50%, such as at least 60%, for example at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 98% of the sucrose in the starting liquid to glucose.

[0114] Step b) may comprise converting at least 50%, such as at least 60%, for example at least 70%, such as at least 80%, for example at least 90%, such as at least 95%, for example at least 98% of the fructose in the starting liquid to glucose.

[0115] When said sugar is converted to glucose with the aid of an enzyme, the amount of sugar converted to glucose may be adjusted by adjusting time of enzyme treatment, the temperature and/or the amount of enzyme used. The skilled person will be able to determine a useful time, temperature and amount to obtain the desired amount of sugar conversion.

[0116] In another embodiment of the disclosure step b) is performed with the aid of a microorganism. Said microorganism should be capable of catabolizing said sugar to form glucose. It is furthermore preferred that said microorganism is capable of excreting at least part of the formed glucose to the surrounding liquid.

[0117] It is furthermore preferred that said microorganism is completely devoid of extracellular proteases, i.e. that said microorganism does not express and excrete any protease.

[0118] In preferred embodiments of the disclosure, then step b) comprises converting maltose to glucose. This is in particular the case in embodiments of the disclosure where the starting liquid comprises maltose and/or maltotriose, for example in embodiments of the disclosure where the starting liquid comprises a malt extract and/or wort. Thus, step may comprise converting maltose to glucose by contacting the starting liquid with a maltose catabolising microorganism capable of converting maltose to glucose.

[0119] Preferably said maltose catabolising microorganism is capable of excreting at least part of said glucose. More preferably said maltose catabolising microorganism is capable of taking up maltose from the starting liquid, hydrolysing said maltose to glucose and excreting at least part of said glucose.

[0120] The maltose catabolising microorganism may be any microorganism, but in one embodiment of the disclosure it is a bacterium. An example of a useful maltose catabolising microorganism is *Lactobacillus sanfransiscensis*.

Incubation with glucose fermenting microorganism

[0121] The methods of the disclosure may comprise a step c) of incubating the liquid with one or more glucose fermenting microorganism.

[0122] The term "glucose fermenting microorganism" as used herein refers to any microorganism capable of converting glucose to alcohols and/or acids under anaerobic conditions. Preferably, the glucose fermenting microorganism is a microorganism capable of converting glucose to an organic acid under anaerobic conditions. Said organic acid may be any of the organic acids described herein below in the section "Organic acid". In particular, the organic acid may be selected from the group consisting of lactic acid, citric acid, malic acid, tartaric acid, acetic acid, succinic acid, isocitric acid, α -ketoglutaric acid, fumaric acid and oxaloacetic acid. In a preferred embodiment of the disclosure the organic acid is lactic acid.

[0123] Accordingly, in a preferred embodiment of the disclosure glucose fermenting microorganism is capable of fermenting glucose to obtain lactic acid. More preferably the glucose fermenting microorganism is capable of taking up glucose, converting the glucose to lactic acid under anaerobic conditions and excreting at least some of said lactic acid.

[0124] The glucose fermenting microorganism to be used with the present disclosure may preferably be selected from the group consisting of yeast and bacteria. In particular, the glucose fermenting microorganism may be a food grade microorganism, i.e. a microorganism which is acceptable for use in production of food and beverages for human beings.

[0125] In one embodiment of the disclosure it is preferred that the glucose fermenting microorganism is a microorganism, which cannot grow to any significant extent in beer, more preferably said microorganism is not capable of growing in beer. In particular, the microorganism may be a bacterium not capable of growing in beer.

[0126] It is furthermore preferred that said microorganism is completely devoid of extracellular proteases, i.e. that said microorganism does not express and excrete any protease. In one embodiment of the disclosure the microorganism is yeast. Said yeast may for example be a yeast of the Kluyveromyces family, e.g. *K. lactis* or *K. marxianus*. The yeast may also be any of the organic acid producing yeasts described in Loureiro V, Malfeito-Ferreira M: Spoilage yeasts in the wine industry, International Journal of Food Microbiology 2003:86:23-50. For example the yeast may be of the *Kloeckera*, *Dekkera/Brettanomyces* or *Pichia* family.

[0127] In one embodiment of the disclosure the yeast may be selected from the group consisting of yeasts listed in table 1.

Table 1

Genus	Species
Ambrosiozyma van der Walt	A. philentoma
	A. platypodis
Cyniclomyces van der Walt et Scott	C. guttulatus
Debaryomyces	D. marama
	D. tamaraii
	D. ranriji var. Vanriji
Guilliermondella Nadson et Krassinikov	G. selenospa
Hanseniaspora zikes	H. guilliermondii
	H. occidentalis
	H. osmophila
	H. uvarum
	H. valbyensis
	H. vineae
Hansenula H. et P. Sydow	H. alni
	H. americana
	H. beckii
	H. beijerinckii
	H. bimundalis

Genus	Species
	H. californica
	H. canadensis
	H. capsulata
	H. dimennae
	H. holstii
	H. jadinii
	H. minuta
	H. petersonii
	H. polymorpha
	H. saturnus var. Saturnus
Issatchenkia Kudriavzev	I. iccidentalis
	I. orientalis
	I. scutulata var. scutulata
	I. terricola
Kluyveromyces van der Walt emend van der Walt	K. delphensis
	K. phaffii
	K. africanus
	K. blattae
	K. waltii
	K. lodderi
	K. polysparus
	K. wickerhomii
	K. aestuarii
Metschnikowia Kamienski	M. bicuspidata var. bicuspidata
	M. lunata
	M. pulcherrima
	M. zobellii
Nadsonia Sydow	N. elongata
Pachysolen Boidin et Adzet	P. tannophilus
Pachytichospora van der Walt	P. transvaalensis
Pichia Hansen	P. abadieae
	P. amylophia

Genus	Species
	P. besseyi
	P. bovis
	P. cactophila
	P. delftensis
	P. dispersa
	P. farinosa
	P. fermentans
Saccharomyces Meyen ex Reess	S. aceti
	S. capensis
	S. chevalieri
	S. coreanus
	S. globosus
	S. norbensis
	S. oleaceus
	S. servazzii
	S. telluris
	S. unisporus
Saccharomyces Hansen Synonym: Saccharomyces ludwigii Hansen var. vini Kroemer et Heinrich (1922)	S. ludwigii
Saccharomycopsis Schönning	S. crataegensis
	S. vini
Schizosaccharomyces Lindner	S. malide vorans
Torulaspora Lindner	T. globosa
Zygosaccharomyces Barkev	Z. bailii
	Z. bisporus
	Z. microellipsoides
	Z. mrakii
Leucosporidium Fell, Stätzell, Hunter et Phaff	L. frigidum
	L. nivalis
Brettanomyces Kufferath et van Laer	B. abstinens
	B. custersianus
	B. naardensis
Candida Berkout	C. anatomiae
	C. apicola
	C. atlantica

Genus	Species
	C. atmospherica
Kloeckera Janke	K. apiculata
	K. apis
	K. Corticis
	K. japonica
	K. javanica
Trichosporon Behrend	T. eriense
	T. fermentans

[0128] In one embodiment of the disclosure the glucose fermenting microorganism is a lactic acid bacterium. The lactic acid bacterium may for example be a bacterium of the order Lactobacillales. In particular the lactic acid bacterium may be a bacterium of a genus selected from the group consisting of *Bifidobacterium*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weisella*. In particular, the lactic acid bacterium may be a bacterium of a genus selected from the group consisting of *Bifidobacterium*, *Lactobacillus*, *Lactococcus* and *Streptococcus*.

[0129] Thus, in one embodiment of the disclosure the glucose fermenting microorganism may be a lactobacillus selected from the group consisting of *L. chungangensis*, *L. fujiensis*, *L. garvieae*, *L. lactis*, *L. piscium*, *L. plantarum* and *L. raffinolactis*. Preferably the glucose fermenting microorganism may be *Lactococcus lactis*.

[0130] Thus, in one embodiment of the disclosure the glucose fermenting microorganism may be a lactobacillus selected from the group consisting of *L. acetotolerans*, *L. acidifarinae*, *L. acidipiscis*, *L. acidophilus*, *L. agilis*, *L. algidus*, *L. alimentarius*, *L. amylolyticus*, *L. amylophilus*, *L. amylotrophicus*, *L. amylovorus*, *L. animalis*, *L. antri*, *L. apodemi*, *L. aviarius*, *L. bifermentans*, *L. brevis*, *L. buchneri*, *L. camelliae*, *L. casei*, *L. catenaformis*, *L. ceti*, *L. coleohominis*, *L. collinoides*, *L. composti*, *L. concavus*, *L. coryniformis*, *L. crispatus*, *L. crustorum*, *L. curvatus*, *L. delbrueckii*, *L. dextrinicus*, *L. diolivorans*, *L. equi*, *L. equigenerosi*, *L. farraginis*, *L. farciminis*, *L. fermentum*, *L. fornicalis*, *L. fructivorans*, *L. frumenti*, *L. fuchuensis*, *L. gallinarum*, *L. gasseri*, *L. gastricus*, *L. ghanensis*, *L. graminis*, *L. hammesii*, *L. hamsteri*, *L. harbinensis*, *L. hayakitensis*, *L. helveticus*, *L. hilgardii*, *L. homohiochii*, *L. iners*, *L. ingluviei*, *L. intestinalis*, *L. jensenii*, *L. johnsonii*, *L. kalixensis*, *L. kefiranoferens*, *L. kefiri*, *L. kimchii*, *L. kitasatonis*, *L. kunkeei*, *L. leichmannii*, *L. lindneri*, *L. malefermentans*, *L. mali*, *L. manihotivorans*, *L. mindensis*, *L. mucosae*, *L. murinus*, *L. nagelii*, *L. namurensis*, *L. nantensis*, *L. oligofermentans*, *L. oris*, *L. panis*, *L. pantheris*, *L. parabrevis*, *L. parabuchneri*, *L. paracollinoides*, *L. parafarraginis*, *L. parakefiri*, *L. paralimentarius*, *L. paraplantarum*, *L. pentosus*, *L. perolens*, *L. plantarum*, *L. pontis*, *L. psittaci*, *L. rennini*, *L. reuteri*, *L. rhamnosus*, *L. rimae*, *L. rogosae*, *L. rossiae*, *L. ruminis*, *L. saerimneri*, *L. sakei*, *L. salivarius*, *L. satsumensis*, *L. secaliphilus*, *L. sharpeae*, *L.*

siliginis, *L. spicheri*, *L. suebicus*, *L. thailandensis*, *L. ultunensis*, *L. vaccinostercus*, *L. vaginalis*, *L. versmoldensis*, *L. vini*, *L. vitulinus*, *L. zeae* and *L. zymae*, preferably the lactobacillus may be selected from the group consisting of *L. amylolyticus*, *L. delbrueckii* and *L. fermentum*.

[0131] Thus, in one embodiment of the disclosure the glucose fermenting microorganism may be a *Pediococcus* selected from the group consisting of *P. acidilactici*, *P. cellicola*, *P. claussenii*, *P. damnosus*, *P. dextrinicus*, *P. ethanolidurans*, *P. inopinatus*, *P. parvulus*, *P. pentosaceus* and *P. stilesii*, preferably the *Pediococcus* may be selected from the group consisting of *P. acidilactici*, *P. dextrinicus* and *P. pentosaceus*.

[0132] In one embodiment of the disclosure the glucose fermenting microorganism may be a *Gluconobacter*, such as *Gluconobacter oxydans*. *Gluconobacter* and in particular *Gluconobacter oxydans* is capable of fermenting a range of sugars to form an organic acid. Thus, for example *Gluconobacter* and in particular *Gluconobacter oxydans* may be capable of fermenting a range of sugars including maltose and glucose to obtain gluconic acid. Thus, in embodiments of the disclosure where the glucose fermenting microorganism is a *gluconobacter*, then step b) may be omitted from the methods of the disclosure. Accordingly, *Gluconobacter* is an example of a microorganism capable of fermenting sugar to form an organic acid.

[0133] The liquid may be incubated with the glucose fermenting microorganism in any suitable manner. In general the incubation is performed in a closed container or a closed vessel. In one preferred embodiment of the disclosure the incubation is performed within one or more chambers defined by two an-ion exchange membranes as described herein below in the section AX-REED.

[0134] The incubation may be performed for any suitable amount of time. In general the incubation may be for in the range from 12 h to 1 week, for example for in the range of 12 h to 48 h, such as for in the range of 12 h to 30 h, for example for in the range of 20 to 28 h. In one embodiment of the disclosure, wherein steps c) and d) are performed simultaneously, the incubation time is selected to obtain a desired retention time. Also the incubation time may be selected in order to obtain a preferred contact time as described herein below in the section "Contact time".

[0135] In particular, the incubation may be performed until a desired glucose level is achieved. For example, step c) may result in a reduction of the glucose level to at the most 100 g/L, preferably to at the most 80 g/L, more preferably to at the most 60 g/L, even more preferably to at the most 40 g/L.

[0136] The incubation may be performed at any suitable temperature. Preferably, the temperature is selected to be an appropriate temperature to allow growth of the particular glucose fermenting microorganism. In general the temperature will be in the range of 15 to 40°C, such as in the range of 20 to 35°C, for example in the range of 23 to 32°C. This may in particular be the case, when the glucose fermenting microorganism is a lactic acid bacterium,

such as *Lactococcus lactis*.

Method of producing beverage with enzymatic conversion of sugar

[0137] It is one aspect of the disclosure to provide methods for producing beverages low in both sugar and alcohol. This is obtained by converting at least part of the sugar in the starting liquid to an organic acid and removal of at least part of the organic acid. The sugar may be converted to an organic acid by the aid of an enzyme. Thus, in one embodiment the disclosure relates to a method comprising the steps of

- a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
- c) incubating said liquid with an enzyme or a mixture of enzymes capable of catalysing conversion of sugar to form an organic acid; and
- d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0138] Frequently, the methods also comprise a step b) of converting any sugar, which is not glucose to glucose. In these embodiments of the disclosure step c) will relate to converting glucose to form an organic acid.

[0139] In the methods of the disclosure step c) is an optional step. However, in one embodiment of the disclosure the methods comprise performing step c). For example, the methods may comprise performing step c (ii). Thus, in one embodiment the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose for example by contacting the starting liquid with an enzyme capable of catalysing conversion the particular sugar in question to glucose.; and
3. c) incubating said liquid with an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to form an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0140] Said enzyme or mixture of enzymes capable of catalysing conversion of glucose to an organic acid may be any of the enzymes or mixtures of enzymes described herein below in the section "Enzymatic conversion of glucose".

[0141] Said AX-REED liquid may be the final beverage or it may be further processed to obtain the final beverage as described below.

[0142] These methods may furthermore comprise step e) and thus in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose for example by contacting the starting liquid with an enzyme capable of catalysing conversion the particular sugar in question to glucose; and
3. c) incubating said liquid with an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to form an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid
wherein said organic is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
5. e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid,
wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0143] As described herein elsewhere, then steps d) and e) may be performed as least partly simultaneous, and thus in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose for example by contacting the starting liquid with an enzyme capable of catalysing conversion the particular sugar in question to glucose; and
3. c) incubating said liquid with an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to form an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid,
wherein said organic is removed through an Anion Exchange Reverse Electro-Enhanced

- Dialysis (AX-REED) membrane stack; and
5. e) at least partly simultaneously removing at least part of one cation from the starting liquid or the partly AX-REED treated liquid, thereby obtaining a REED liquid, wherein said REED liquid retains at least 65% of said at least one micro-nutrient, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0144] The CX-REED liquid or the REED liquid may be the final beverage or it may be further processed to obtain the final beverage. It is preferred that the CX-REED liquid or the REED liquid is the final beverage or that the final beverage is obtained by adding one or more additional compounds to the CX-REED liquid or the REED liquid.

[0145] Thus, the method may furthermore comprise a step f) of adding one or more additional compounds to the starting liquid, the AX-REED liquid, the CX-REED liquid or the REED liquid. In embodiments of the disclosure not containing step e), then step f) preferably comprises adding one or more additional compounds to the AX-REED liquid. In one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose for example by contacting the starting liquid with an enzyme capable of catalysing conversion the particular sugar in question to glucose; and
3. c) incubating said liquid with an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to form an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
5. e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack; and
6. f) adding one or more additional compounds to the CX-REED liquid, preferably one or more additional compound selected from the group consisting of flavour compounds and preservatives, thereby obtaining the beverage.

[0146] The method may also comprise a step g) of adding one or more additional liquids to the AX-REED liquid or the CX-REED liquid or the REED liquid in order to obtain the final beverage. In particular said additional liquids may be beverages, so that the final beverage is a mixture between the CX-REED liquid and an additional beverage or a mixture between the REED liquid

and an additional beverage. Thus, in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose for example by contacting the starting liquid with an enzyme capable of catalysing conversion the particular sugar in question to glucose; and
3. c) incubating said liquid with an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to form an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
5. e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack; and
6. f) optionally adding one or more additional compounds to the CX-REED liquid, preferably one or more additional compound selected from the group consisting of flavour compounds and preservatives; and
7. g) providing an additional liquid, for example a beverage and mixing said CX-REED liquid with said additional liquid, thereby obtaining the beverage.

[0147] Said additional liquid may be any of the additional liquids described herein above in the section "Method of producing fermented beverages". The methods may be useful for preparing low alcohol beer, low alcohol cider, low alcohol wine and low calorie cider as described above in the section "Method of producing fermented beverages".

[0148] It is also comprised within the present disclosure that the methods may comprise a step h) of further processing the AX-REED liquid or the CX-REED liquid or the REED liquid in order to obtain the final beverage. Said further processing may for example be a step of incubation of the AX-REED liquid or the CX-REED liquid or REED liquid with one or more microorganisms, such as yeast. Said methods may comprise above described step g), and thus the methods may comprise incubation of the liquid obtained in step g) with one or more microorganisms, such as yeast. The microorganisms to be employed in step h) may in particular be yeast, such as Brewer's yeast, for example yeast *Saccharomyces cerevisiae* or *Saccharomyces pastorianus*, formerly known as *S. carlsbergensis*. Thus, in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

- a) providing a starting liquid comprising at least one micro-nutrient and at least one sugar; and
- b) if said sugar is not glucose converting at least some of said sugar to glucose; and

- c) incubating said liquid with an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to form an organic acid; and
- d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and
- e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack; and
- f) optionally adding one or more additional compounds to the CX-REED liquid, preferably one or more additional compound selected from the group consisting of flavour compounds and preservatives; and
- h) incubating said AX-REED liquid or said X-REED liquid with one or more microorganisms, such as yeast, optionally followed removal of said microorganism, thereby obtaining the beverage.

[0149] During step c) an organic acid is generated and during step d) at least some of said generated organic acid is removed. As described elsewhere steps c) and d) may be performed simultaneously, and thus the organic acid may be continuously generated with the aid of said enzyme(s) and at least some of the generated organic acid may continuously be removed through said AX-REED membrane stack. While at least 10% of the organic acid is removed it is contained within the disclosure that at least some of the generated organic acid is maintained in the beverage and thus it is comprised within the disclosure, that the beverage may contain more organic acid than the starting liquid. This is in particular the case, when the starting liquid has a high level of sugar and a low level of organic acids. In order to reach a palatable ratio between sugar and organic acid, such as any of the ratios described herein below in the section "Ratio of sugar to organic acid", then it may be preferable that the beverage contains more organic acid than the starting liquid. Thus, preferably the beverage has a ratio of sugar to organic acid as described herein below in the section "Ratio of sugar to organic acid".

[0150] Organic acids may inhibit the activity of certain enzymes. Thus, in embodiments of the disclosure where step c) and step d) are performed simultaneously, it is preferred that the sufficient organic acid is removed in order for the enzyme(s) to maintain at least most of the activity.

[0151] It is preferred that at least 10% of the generated organic acid is removed during step d). Thus for example at least 15%, for example at least 20%, such as at least 25%, such as at least 30% of the organic acid generated in step c) is preferably removed in step d). Said organic acid may be any of the organic acids described herein below in the section "Organic acid".

[0152] In particular it is preferred that at least 10% of generated gluconic acid is removed during step d), Thus, step d) may in a preferred embodiment of the disclosure comprise removal of at least 10% of the gluconic acid, for example at least 15% of the gluconic acid, for example at least 20% of the gluconic acid, such as at least 25% of the gluconic acid, such as at least 30% of the gluconic acid generated in step c). This is in particular the case in embodiments of the disclosure, where said enzyme or mixture of enzymes catalyses conversion of glucose to form gluconic acid.

[0153] In particular it is preferred that at least 10% of generated lactic acid is removed during step d), Thus, step d) may in a preferred embodiment of the disclosure comprise removal of at least 10% of the lactic acid, for example at least 15% of the lactic acid, for example at least 20% of the lactic acid, such as at least 25% of the lactic acid, such as at least 30% of the lactic acid generated in step c). This is in particular the case in embodiments of the disclosure, where said enzyme or mixture of enzymes catalyses conversion of glucose to form lactic acid.

[0154] In these embodiments of the disclosure the micro-nutrient may for example be any of the micro-nutrients described herein below in the section "Micro-nutrient"; and the sugar may for example be any of the sugars described in the section "Sugar", and step d) may for example be performed in any of the ways described in the section "AX-REED and step e) may for example be performed in any of the ways described in the section "CX-REED". Said ratio of sugar to organic acid may be any of the ratios described herein below in the section "Ratio of sugar to organic acid".

[0155] In particular, in embodiments of the disclosure relating to methods of producing beverages with enzymatic conversion of sugar, then it is preferred that said micro-nutrient is selected from the group consisting of minerals and vitamins. Similarly, in embodiments of the disclosure relating to methods pf producing beverages with enzymatic conversion of glucose, then it is preferred that said micronutrient is selected from the group consisting of minerals and vitamins, and in particular from the group consisting of calcium, magnesium, iron, vitamin B1 and vitamin B2. In particular the micro-nutrient may be selected from the group of minerals and vitamins described herein below in the section "Micro-nutrient".

[0156] The starting liquid may be any liquid useful for preparing a beverage. It is generally preferred that the starting liquid is a natural product. The term "natural product" as used herein refers to a product obtain from natural sources by extraction in water or by squeezing, wherein no extra chemicals are added. Thus, in one embodiment of the disclosure the starting liquid is an extract, concentrate or a juice of a plant or a plant part, where no extra sugar has been added.

[0157] In embodiments of the disclosure relating to producing a beverage with enzymatic conversion of sugar, it is preferred that the starting liquid has a relatively high level of one or more sugars, for example a high level of one or more of the sugars described herein below in the section "Sugar". Thus, the starting liquid may for example comprise more than 10%, for

example more than 9%, such as more than 8%, for example more than 7% sugar. Said percentages are given as w/w.

[0158] In one embodiment of the disclosure the starting liquid is a liquid with a high level of maltose. Thus the starting liquid may be a liquid containing more than 40 g/L, such as more than 50 g/L, for example more than 60g/L maltose.

[0159] In particular, the starting liquid may be an extract of one or more cereals. Said cereals may for example be selected from the group consisting of barley, wheat, rye, oat, maize, rice, sorghum, millet, triticale, buckwheat, fonio and quinona. More preferably, the cereal is selected from the groups consisting of barley, wheat, rye, oat, maize and rice, more preferably the cereal is barley.

[0160] The extract may be an extract of the cereal per se, however the cereal may also be malted and the extract may be an extract of the malted cereal. As used herein the term "malted", refers to the cereal grains having been subjected to steeping, allowed to germinate and then dried. Said drying may for example be kiln drying.

[0161] Said extract of a cereal or a malted cereal is preferably an aqueous extract.

[0162] Thus, in a preferred embodiment of the disclosure, and in particular in embodiments of the disclosure relating to methods of producing a beverage with enzymatic conversion of sugar, then the starting liquid may be a malt extract, such as a barley malt extract. More preferably the starting liquid may be wort. The starting liquid may also be an extract of a mixture or barley malt and other cereals.

[0163] In a particularly preferred embodiment of the disclosure the starting liquid is wort. By the term "wort" as used herein is meant a liquid extract of malt. Wort may also be prepared by incubating an extract of un-malted barley with an enzyme mixture that hydrolyzes the barley components. In addition to said malt or barley-derived extracts, the wort may be prepared from malt and additional components, such as additional starch-containing material partly converted into fermentable sugars. The wort is in general obtained by mashing, optionally followed by sparging.

[0164] The term "Mashing" as used herein refers to the incubation of milled malt in water. Mashing is preferably performed at a specific temperature, and in a specific volume of water. Mashing can occur in the presence of adjuncts, which is understood to comprise any carbohydrate source other than malt, such as, but not limited to, unmalted barley, barley syrups, or maize, or rice - either as whole kernels or processed products like grits, syrups or starch.

[0165] The term "sparging" as used herein refers to a process of extracting residual sugars and other compounds from spent grains after mashing with hot water. Sparging is typically conducted in a lauter tun, a mash filter, or another apparatus to allow separation of the

extracted water from spent grains.

[0166] The wort obtained after mashing is generally referred to as "first wort", while the wort obtained after sparging is generally referred to as the "second wort". If not specified, the term wort may be first wort, second wort, or a combination of both.

[0167] Thus, the starting liquid may be wort, such as first wort, second wort or a mixture thereof.

[0168] It is also comprised within the disclosure that the starting liquid may be an extract, a concentrate or a juice of a plant or a plant part, which has been treated with one or more enzymes. For example said extract, concentrate or juice of a plant or a plant part, may have been treated with one or more enzymes selected from the group consisting of glucosidases, proteases, pectinases and cellulases. In embodiments of the disclosure, where the starting liquid is wort, the said starting liquid may have been prepared employing a step of enzyme treatment with one or more enzymes selected from the group consisting of glucosidases, proteases, pectinases and cellulases, preferably from the group consisting of glucan 1,4- α -glucosidases, proteases, pullulanases, α -amylases, β -amylases, limit dextrinases and β -glucosidases. Treatment with glucan 1,4- α -glucosidases may be considered as step b) of the methods of the disclosure. Said enzymes may in particular be employed in order to degrade starch in order to obtain sugars, such as maltose.

[0169] However in one embodiment of the disclosure it is preferred that no proteases are added at any step during the procedure.

[0170] In certain embodiments of the disclosure the pH of the starting liquid may be adjusted by addition of base or acid, such as potassium hydroxide or lactic acid. This may for example be done in order to start the enzyme treatment at a pH where the enzyme has good activity.

[0171] The methods of the disclosure may thus comprise several enzyme treatments, e.g. one or more of the following:

1. i) Treatment with one or more enzymes to obtain a starting liquid comprising sugar. Said enzymes may in particular be enzymes capable of catalysing conversion of starch to obtain sugar. Useful examples of such enzymes are described herein above in this section.
2. ii) Treatment with one or more enzymes capable of catalysing conversion of other sugars to glucose, e.g. enzymes capable of catalysing conversion of maltose to glucose. This enzyme treatment may constitute step b) of the methods of the disclosure. Useful examples of such enzymes are described herein above in the section "Converting sugar to glucose".
3. iii) Treatment with an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to form an organic acid. This enzyme treatment may constitute step c). Useful examples of such enzymes are described herein below in the section "Enzymatic

conversion of glucose".

[0172] These enzyme treatments may be performed simultaneously or sequentially. In particular treatment ii) and iii), which may constitute steps b) and c) of the methods of the disclosure may be performed simultaneously.

[0173] In one embodiment of the disclosure the starting liquid is a fruit juice or extract containing a high level of sugar. In particular, the starting liquid may be a fruit juice or extract, which naturally contains a high level of sugar, such as more than 40 g/L, such as more than 50 g/L, for example more than 60g/L sugar. Said fruit juice may for example be grape juice, pear juice or apple juice. The methods of the disclosure may be used to produce a palatable beverage with reduced sugar content compared to the fruit juice or extract, while retaining one or more valuable micronutrients.

[0174] In embodiments of the disclosure, wherein the starting liquid comprises a malt extract and/or wort, then the starting liquid in general will comprise high levels of maltose, and in these embodiments of the disclosure the methods preferably comprises a step of converting at least some of said maltose to glucose.

[0175] Accordingly, it is also one aspect of the disclosure to provide methods of preparing a beverage, wherein the methods comprise the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient and maltose, wherein said starting liquid for example may comprise or even consist of a malt extract and/or wort; and
2. b) converting at least some of said maltose to glucose for example by incubation with an enzyme capable of catalysing hydrolysis of maltose to glucose; and
3. c) incubating said liquid with an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to form an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid,
wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack as described herein below in the section "AX-REED"

and optionally the method further comprises one of both of the steps e) and f) as outlined above.

[0176] Step b) of converting maltose to glucose may be performed in any of the ways described above in the section "Converting sugar to glucose", and in particular step b) may comprise contacting the starting liquid with an enzyme capable of catalysing hydrolysis of maltose to glucose as described in the section "Converting sugar to glucose",

[0177] Step c) of the methods for producing a beverage with enzymatic conversion of glucose may be performed in any of the ways described herein below in the section "Enzymatic conversion of glucose".

[0178] Step b) and step c) may be performed sequentially, by first performing step b) and then step c). It is however also comprised in the disclosure that step b) and step c) can be performed simultaneously or at least partly simultaneously. For example, when step b) is performed with the aid of an enzyme as described in the section "Converting sugar to glucose" herein below, then the enzyme may be added to the starting liquid together with said enzyme or mixture of enzymes capable of catalysing conversion of glucose to form an organic acid. When step b) is performed with the aid of a microorganism such as a maltose catabolising microorganism as described in the section "Converting sugar to glucose" herein below, then the starting liquid may be incubated with said microorganism and with said enzyme or mixture of enzymes capable of catalysing conversion of glucose to obtain an organic acid simultaneously. In embodiments of the disclosure where step b) is performed with the aid of a microorganism it is preferred that step b) and step c) are performed simultaneously.

[0179] Steps c) and d) may also be performed sequentially, by first performing step c) and then step d). It is however preferred that step c) and step d) are performed simultaneously or at least partly simultaneously. Thus, the liquid may be incubated with said enzyme or mixture of enzymes capable of catalysing conversion of glucose to form an organic acid and simultaneously at least some of said organic acid is removed from the liquid. Thus, as the organic acid is produced by the enzymatically catalysed reaction, then it is removed from the liquid, ensuring a constant low level of said organic acid in the liquid. This may avoid reduction or inhibition of enzyme activity due to high product levels and/or due to acidic conditions.

[0180] Accordingly, the steps b), c) and d) may all be performed simultaneously. Alternatively, step b) may be performed first, and then steps c) and d) may be performed simultaneously.

[0181] Step d) may be performed prior to step e), however it is also contained within the disclosure that steps d) and e) may be performed simultaneously.

[0182] In one preferred embodiment of the disclosure steps d) and e) are performed simultaneously. Thus, in this embodiment of the disclosure steps b), c), d) and e) may all be performed simultaneously. Alternatively, step b) may be performed first and then steps c), d) and e) may be performed simultaneously. Steps d) and e) may in particular be performed simultaneously by using a REED equipment containing at least one AX-REED and at least one CX-REED membrane stack, wherein said AX-REED and said CX-REED membrane stacks are connected in parallel.

[0183] In yet another very preferred embodiment of the disclosure steps d) and e) are performed partly simultaneously. In this embodiment of the disclosure step d) may for example be performed for a given time period, where after both steps d) and e) are performed

simultaneously. Thus, one or more acidic ions may be removed from the liquid through AX-REED for a given time period, where after both acidic ions and at least one cation are removed respectively through AX-REED and CX-REED, wherein the AX-REED and the CX-REED are performed simultaneously. Thus, in this embodiment of the disclosure steps b), c), d) and e) may all be performed at least partly simultaneously. Alternatively, steps b) may be performed first and then steps c), d) and e) may be performed at least partly simultaneously. When steps d) and e) are performed at least partly simultaneously, this is preferably done by using a REED equipment containing at least one AX-REED and at least one CX-REED membrane stack, wherein said AX-REED and said CX-REED membrane stacks are connected in parallel.

[0184] In one embodiment of the disclosure it is preferred that no sugar is added to starting liquid and furthermore, it is preferred that no sugar is added at any step during the method. In addition it is preferred that no sugar is added to the final beverage.

[0185] In some embodiments of the disclosure, the methods do not comprise a step b), but rather comprises a step c) where any sugar is converted to form an organic acid with the aid of enzymes. Thus the methods may comprise a step c) of incubating the starting liquid with an enzyme or a mixture of enzymes capable of catalysing conversion of sugar to form an organic acid. Said enzyme or mixture of enzymes may for example be an enzyme or mixture of enzyme capable of catalyzing conversion of maltose to form an organic acid. In particular, the enzyme or mixture of enzymes may be capable of catalyzing conversion of maltose to form maltobionic acid. This is in particular the case in embodiments of the disclosure, where the starting liquid comprises a high level of maltose, e.g. when the starting liquid comprise or even consists of a cereal extreact, e.g. when the starting liquid is wort.

[0186] It is comprised within the disclosure that any of the methods may comprise as a last step, the step i) of adding CO₂ in order to obtain a carbonated beverage.

Enzymatic conversion of glucose

[0187] In embodiments of the disclosure relating to methods of producing a beverage with enzymatic conversion of glucose, the methods of the disclosure comprises a step of incubating a liquid with an enzyme or a mixture of enzymes capable of catalyzing conversion of glucose to form an organic acid.

[0188] Said liquid may be the starting liquid. Said liquid may also be a liquid obtained after the starting liquid has been treated to convert at least some of the sugar, which is not glucose into glucose. Said liquid may also be the starting liquid, which simultaneous with incubation with an enzyme or a mixture of enzymes capable of catalyzing conversion of glucose to form an organic acid, also is treated to convert at least some of the sugar, which is not glucose into glucose.

[0189] Said enzyme or a mixture of enzymes capable of catalyzing conversion of glucose to

form an organic acid may comprise any enzymes, which are capable of catalyzing conversion of glucose to form an organic acid.

[0190] In one preferred embodiment of the disclosure the enzyme or a mixture of enzymes capable of catalyzing conversion of glucose to form an organic acid comprises or even consists of a glucose oxidase.

[0191] The glucose oxidase to be used with the present disclosure is in general an enzyme classified under EC 1.1.3.4. Thus the glucose oxidase to be used with the present disclosure is an oxido-reductase that is capable of catalysing the oxidation of glucose to hydrogen peroxide and D-glucono- δ -lactone. This in particular, the glucose oxidase to be used with the present disclosure is an enzyme capable of of catalysing the following reaction



[0192] D-glucono-1,5-lactone hydrolyses in water to gluconic acid. Thus, in an aqueous environment the conversion of glucose catalysed by glucose oxidase lead to the formation of gluconic acid. In the methods of the disclosure the enzyme or mixture of enzymes capable of catalyzing conversion of glucose to form an organic acid are in general employed in an aqueous environment and thus the enzyme or mixture of enzymes capable of catalyzing conversion of glucose to form an organic acid may comprise or even consist of glucose oxidase.

[0193] The glucose oxidase may be any useful glucose oxidase. For example the glucose oxidase may be glucose oxidase of *Aspergillus niger* or *Penicillium amagasakiense*. In one embodiment of the disclosure the glucose oxidase is a glucose oxidase of SEQ ID NO: 10 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. The glucose oxidase may also comprise or even consist of aa 23-605 of SEQ ID NO:10 or a functional homologue of aa 23-605 of SEQ ID NO:10 sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. The glucose oxidase may also be a glucose oxidase of SEQ ID NO: 11 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. A functional homologue of a given glucose oxidase is a polypeptide capable of catalyzing the reaction:



and which have the aforementioned sequence identity with a reference glucan 1,4- α -glucosidase.

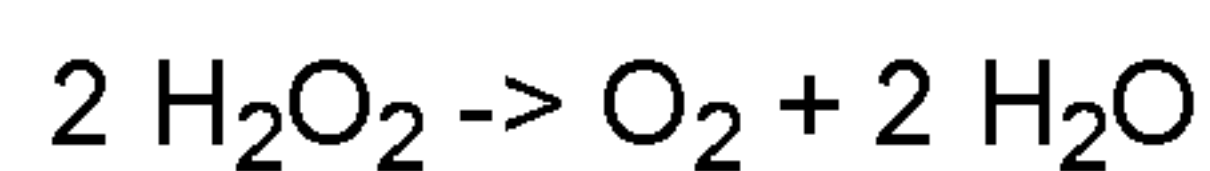
[0194] The glucose oxidase may also be one of commercially available glucose oxidases, such

as Hyderase available from Amano Pharmaceutical Co. Ltd., Nagoya, Japan. An advantage of the Hyderase is that it also comprises catalase activity. Thus, Hyderase contains both glucose oxidase and catalase activity.

[0195] Furthermore, glucose oxidases described in GB1,373,562, US4,675,191 and US 20120114791 may be used with the present disclosure.

[0196] As noted above the reaction catalyzed by glucose oxidase also may lead to the formation of H₂O₂. In general, it is not desirable that beverages contain high levels of H₂O₂ and thus, in embodiments of the disclosure involving use of glucose oxidase, it is preferred that the methods also comprise a step of removing H₂O₂. For example the enzyme or mixture of enzymes capable of catalyzing conversion of glucose to form an organic acid may also contain a catalase activity.

[0197] The catalase to be used with the present disclosure may be any enzyme capable of catalyzing the decomposition of hydrogen peroxide to water and oxygen. Thus, the catalase may be an enzyme classified under EC 1.11.1.6. In particular the catalase may be an enzyme catalyzing the following reaction:



[0198] The catalase may be any useful catalase. For example the catalase may be catalase from *Aspergillus niger*, *Bacillus subtilis* or *Bos taurus* (in particular, from liver of *Bos taurus*). In one embodiment of the disclosure the catalase is a catalase of SEQ ID NO: 12 or a functional homologue thereof sharing at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity therewith. A functional homologue of a catalase is a polypeptide capable of catalyzing the reaction:



and which have the aforementioned sequence identity with a reference glucan 1,4- α -glucosidase.

[0199] It is to be understood that any glucose oxidase can be used in the method according to the disclosure, provided that the glucose oxidase exhibits a reasonable activity and stability at the pH and temperature prevailing during conversion of the glucose. Thus, both soluble and immobilized glucose oxidase preparations may be used, even if soluble glucose oxidase preparations are usually preferred. The same considerations are valid for catalase, if used.

[0200] It is noted here that aerating the liquid, e.g. the starting liquid with oxygen is advantageous during conversion of glucose to gluconic acid. The aerating has not been found

to be detrimental to the taste of the beverage.

[0201] The temperature during enzymatic conversion of the glucose may for example be between 10°C and 40°C, preferably between 10°C and 30°C.

[0202] As described herein above in the section "Method of producing a beverage with enzymatic conversion of sugar", then the gluconic acid formed may be continuously removed through AX-REED. Thus, the reaction is not inhibited by accumulation of gluconic acid.

[0203] In a preferred embodiment of the method according to the disclosure oxygen is supplied continuously to the liquid incubated with the enzyme or mixture of enzymes containing a glucose oxidase. The supply of oxygen has a remarkably beneficial influence on the reaction rate of the enzymatic reaction. Thus, continuous introduction of oxygen ensures a high reaction rate. The oxygen may be supplied by any suitable means, for example oxygen may be supplied by means of an air pump, a most efficient means for introducing oxygen into the liquid.

[0204] The enzyme or mixture of enzymes may in addition to the glucose oxidase and the catalase also contain additional enzymatic activities. However, it is generally preferred that no glucose isomerase is added to any of the liquids during the methods of the disclosure. Thus it is preferred that no enzyme classified under EC 5.3.1.5 is added to any of the liquid during the methods of the disclosure.

[0205] Also it is preferred that gluconic acid generated during the methods of the disclosure is removed by AX-REED rather than by precipitation. Thus, it is preferred that the methods of the disclosure do not comprise addition of a substance forming a sparingly soluble salt of gluconic acid, such as calcium carbonate.

[0206] In certain embodiments of the disclosure the AX-REED liquid or the CX-REED liquid or the REED liquid may be subjected to a fermentation. In some embodiments of the disclosure however; it is preferred that neither the AX-REED liquid nor the CX-REED liquid nor the REED liquid is subjected to an alcohol fermentation. Thus, in some embodiments, the methods of the disclosure do not comprise a step of fermentation generating alcohol.

[0207] The enzymatic conversion of glucose is preferably undertaken in manner so that sufficient glucose is removed from the liquid. This may be obtained for example by adjusting the amount of enzyme and/or the incubation time. The skilled person will be able to select a suitable amount of enzyme and a suitable incubation time. In particular, it is preferred that step c) of the methods of the disclosure result in a reduction of the glucose level of at least 20%, preferably of at least 30%, more preferably of at least 40%, even more preferably of at least 50%, yet more preferably of at least 60%, even more preferably of at least 70%.

Method of producing a beverage without bacterial fermentation

[0208] In one embodiment the present disclosure relates to methods for producing a beverage without bacterial fermentation. Said methods in general comprise only a step of removing organic acids from a starting liquid to obtain a beverage. Accordingly, the methods of this embodiment of the disclosure are mainly applicable for starting liquids, which contain organic acid, i.e. which contain a high level of one or more organic acids.

[0209] Thus, in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

- a) providing a starting liquid comprising at least one micro-nutrient and at least one organic acid; and
- d) removing at least 10% of at least one organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0210] In general the starting liquid has at least some sweetness and thus, the starting liquid frequently contains sugar. For example the starting liquid may contain glucose. Thus. The disclosure also provides methods of producing a beverages, wherein the methods comprises the steps of

- a) providing a starting liquid comprising at least one micro-nutrient, at least one organic acid and glucose; and
- d) removing at least 10% of at least one organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0211] Said AX-REED liquid may be final beverage or it may be further processed into the final beverage.

[0212] This method may furthermore comprise step e) and thus in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

- a) providing a starting liquid comprising at least one micro-nutrient, at least one organic acid and glucose; and
- d) removing at least 10% of at least one organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced

Dialysis (AX-REED) membrane stack; and

e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0213] Said CX-REED liquid may be the final beverage or it may be further processed in order to obtain the final beverage.

[0214] As described herein elsewhere steps d) and e) may be performed at least partly simultaneously. Thus in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of

a) providing a starting liquid comprising at least one micro-nutrient, at least one organic acid and glucose; and

d) removing at least 10% of said organic acid from said liquid, wherein said organic is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and

e) at least partly simultaneously removing at least part of one cation from the starting liquid or the partly AX-REED treated liquid, thereby obtaining a REED liquid, wherein said REED liquid retains at least 65% of said at least one micro-nutrient, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0215] Said REED liquid may be the final beverage or it may be further processed in order to obtain the final beverage.

[0216] Thus, this method may also contain an additional step f) of adding one or more additional compounds to the starting liquid, the AX-REED liquid or to the CX-REED liquid or to the REED liquid. In embodiments of the disclosure not containing step e), then it is preferred that the additional compound(s) are added to the AX-REED liquid. The method may in one embodiment of the disclosure comprise the steps of:

a) providing a starting liquid comprising at least one micro-nutrient, at least one organic acid and glucose; and

d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack

e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack; and

f) adding one or more additional compounds, preferably adding one or more flavour compounds and/or preservatives, thereby obtaining the beverage.

[0217] The method may also comprise a step g) of adding one or more additional liquids to the AX-REED liquid or the CX-REED liquid or the REED liquid in order to obtain the final beverage. In particular said additional liquids may be beverages, so that the final beverage is a mixture between the CX-REED liquid and an additional beverage or a mixture between the REED liquid and an additional beverage. Thus, in one aspect the disclosure relates to a method of preparing a beverage, wherein the method comprises the steps of:

a) providing a starting liquid comprising at least one micro-nutrient, at least one organic acid and glucose; and

d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining an AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack

e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a CX-REED liquid wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack; and

f) optionally adding one or more additional compounds, preferably adding one or more flavour compounds and/or preservatives; and

g) providing an additional liquid, for example a beverage e.g. a fruit juice and mixing said CX-REED liquid with said additional liquid, thereby obtaining the beverage

[0218] In these embodiments of the disclosure the starting liquid already contains organic acid and step d) relates to removing at least some of said organic acid, thereby reducing the acid content of the starting liquid to obtain a palatable beverage. Preferably the beverage has a ratio of sugar to organic acid as described herein below in the section "Ratio of sugar to organic acid", and accordingly it is preferred that sufficient organic acid is removed in order to reach said ratio.

[0219] Thus, preferably at least 10% of at least one organic acid, for example at least 15% of

at least one organic acid, for example at least 20% of at least one organic acid, such as at least 25% of at least one organic acid, such as at least 30% of at least one organic acid is removed during step d). More preferably at least 10% of at least two organic acids, for example at least 15% of at least two organic acids, for example at least 20% of at least two organic acids, such as at least 25% of at least two organic acids, such as at least 30% of at least two organic acids are removed during step d). For example at least 10% of all organic acids, for example at least 15% of all organic acids, for example at least 20% of all organic acids, such as at least 25% of all organic acids, such as at least 30% of all organic acids are removed during step d).

[0220] In one embodiment of the disclosure it is preferred that at least 10% of citric acid, for example at least 15% of citric acid, for example at least 20% of citric acid, such as at least 25% of citric acid, such as at least 30% of citric acid is removed during step d). This is in particular the case, when the starting liquid comprises citric acid.

[0221] In another embodiment of the disclosure it is preferred that at least 10% of malic acid, for example at least 15% of malic acid, for example at least 20% of malic acid, such as at least 25% of malic acid, such as at least 30% of malic acid is removed during step d). This is in particular the case, when the starting liquid comprises malic acid.

[0222] Said micro-nutrient may be any of the micro-nutrients described herein below in the section "Micro-nutrients". In particular, in embodiments of the disclosure relating to methods of producing beverages without bacterial fermentation, then it is preferred that said micro-nutrient is selected from the group consisting of minerals, vitamins and antioxidants and in particular from the group of minerals, vitamins and antioxidants described herein below in the section "Micro-nutrient".

[0223] The starting liquid in this embodiment of the disclosure is any liquid useful for preparing a beverage. It is preferred that the starting liquid is a liquid, which is useful as a beverage apart from it having a too high content of organic acids. It is generally preferred that the starting liquid is a natural product. The term "natural product" as used herein refers to a product obtained from natural sources by extraction in water or by squeezing, wherein no extra chemicals are added. Thus, in one embodiment of the disclosure the starting liquid is an extract or a juice of a plant or a plant part, where no extra sugar has been added.

[0224] Thus in one embodiment of the disclosure the starting liquid is a fruit juice, such as a juice of a citrus fruit. The citrus fruit may for example be selected from the group consisting of orange, lime, pomelo, lemon, mandarin, satsuma, grape fruit, Australian lime and kumquats. In one preferred embodiment of the disclosure the starting liquid is lemon juice. The starting liquid may also be a berry juice, such as black currant juice. The starting liquid may also be a vegetable juice, such as tomato juice or carrot juice.

[0225] In another embodiment of the disclosure the starting liquid is a fermented fruit juice. For example the starting liquid may be selected from the group consisting of fermented apple juice

and fermented pear juice.

[0226] In yet another embodiment of the disclosure the starting liquid is an extract of a fruit, for example the starting liquid may be an extract of a fruit selected from the group consisting of rosehip, sloe, blackcurrant and crowberry.

[0227] It is also comprised within the disclosure that the starting liquid may be an extract, a concentrate or a juice of a plant or a plant part, which has been treated with one or more enzymes. For example said extract, concentrate or juice of a plant or a plant part, may have been treated with one or more enzymes selected from the group consisting of glucosidases, proteases, pectinases and cellulases. In embodiments of the disclosure, where the starting liquid is a fruit juice, a fruit extract or a fruit concentrate then the starting liquid may have been prepared employing a step of enzyme treatment with one or more enzymes selected from the group consisting of pectinases and cellulases.

[0228] The starting liquid may also be a mixture of one or more of the aforementioned juices, extract and concentrates.

[0229] The organic acid may be any organic acid contained within the starting liquid. In particular the organic acid may be any of the organic acids described herein below in the section "Organic acid". Preferably, the organic acid may be selected from the group consisting of lactic acid, citric acid, malic acid, tartaric acid, acetic acid, succinic acid, isocitric acid, α -ketoglutaric acid, fumaric acid and oxaloacetic acid. In embodiments of the disclosure where the starting liquid is a citrus fruit juice, then the organic acid may in particular be citric acid. In embodiments of the disclosure where the starting liquid is fermented apple juice or fermented pear juice, then the organic acid may in particular be malic acid.

[0230] As described herein above it is one advantage of the methods of the disclosure that the beverages prepared according to the methods are low in calories, and in particular are low in sugar. Thus, in embodiments of the disclosure which does not comprise a fermentation step, then it is preferred that the starting liquid does not have a high level of sugar. Thus, it is preferred that the starting liquid contains at the most 10%, preferably at the most 9%, yet more preferably at the most 8%, for example at the most 7% sugar. More preferably, the combined concentration of glucose, fructose, maltose, lactose and sucrose in the starting liquid is at the most 10%, preferably at the most 9%, yet more preferably at the most 8%, for example at the most 7%. It is also preferred that the starting liquid contains at the most 10%, preferably at the most 9%, yet more preferably at the most 8%, for example at the most 7% glucose. Said percentages are all provided as w/w.

[0231] The ratio of sugar to organic acid may be any of the ratios described herein below in the section "Ratio of sugar to organic acid".

[0232] Step f) comprising adding one or more additional compounds. Said additional compounds may preferably be selected from the group consisting of sugars, flavour

compounds, preservatives and water.

[0233] It is preferred that the beverages prepared by the methods of the disclosure are low in sugar, and thus in embodiments of the disclosure, where sugar is added it is preferred that the resulting beverages contain at the most 10%, preferably at the most 9%, yet more preferably at the most 8%, for example at the most 7% sugar, preferably at the most 10%, preferably at the most 9%, yet more preferably at the most 8%, for example at the most 7% glucose.

[0234] The flavour compound may be any of the flavour compounds described herein below in the section "flavour compounds".

[0235] Step d) of the methods described in this section may be performed in any of the ways described herein below in the section "AX-REED".

[0236] Step e) of the methods described in this section may be performed in any of the ways described herein below in the section "CX-REED".

[0237] Steps d) and e) may be performed simultaneously or sequentially, however preferably step d) is performed prior to step e).

[0238] In one preferred embodiment of the disclosure steps d) and e) are performed simultaneously. Steps d) and e) may in particular be performed simultaneously by using a REED equipment containing at least one AX-REED and at least one CX-REED membrane stack, wherein said AX-REED and said CX-REED membrane stacks are connected in parallel.

[0239] In yet another very preferred embodiment of the disclosure steps d) and e) are performed partly simultaneously. In this embodiment step d) may for example be performed for a given time period, where after both steps d) and e) are performed simultaneously. In particular step d) may be performed until a predetermined pH desirable for the final beverage is achieved, where after both steps d) and e) may be performed simultaneously. Thus, one or more acidic ions may be removed from the liquid through AX-REED for a given time period, where after both acidic ions and at least one cation are removed respectively through AX-REED and CX-REED, wherein the AX-REED and the CX-REED are performed simultaneously. When AX-REED is performed alone, acidic ions are continuously removed thereby increasing the pH. When AX-REED and CX-REED are performed in parallel, then preferably the pH may be maintained relatively stable. Thus, AX-REED may be performed until at desired pH is reached, whereafter AX-REED and CX-REED may be performed in parallel until a desired conductivity is reached. When steps d) and e) are performed at least partly simultaneously, this is preferably done by using a REED equipment containing at least one AX-REED and at least one CX-REED membrane stack, wherein said AX-REED and said CX-REED membrane stacks are connected in parallel. Accordingly, in one embodiment of the disclosure provides methods for preparing a beverage comprising the steps of

a) providing a starting liquid (e.g. a fruit juice or a fruit extract) comprising at least one micro-

nutrient, at least one organic acid and glucose; and

d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid,

wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack

and wherein said process is continued until a predetermined pH is reached,

e) simultaneously removing at least part of one organic acid and at least part of one cation from the liquid obtained in step d), while retaining at least 65% of said at least one micro-nutrient in said liquid, thereby obtaining a REED liquid wherein

said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack; and

said cation is removed through a Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack; and

wherein said process is continued until a predetermined conductivity is reached,

f) optionally adding one or more additional compounds, preferably adding one or more flavour compounds and/or preservatives; and

g) optionally providing an additional liquid, for example a beverage e.g. a fruit juice and mixing said CX-REED liquid with said additional liquid.

[0240] Said predetermined pH is preferably selected in order to achieve a final beverage with a desirable ratio of sugar to organic acid. In particular, said ratio of sugar to organic acid may be any of the ratios of sugar to organic acid described herein below in the section "Ratio of sugar to organic acid". In embodiments of the disclosure relating to relatively acidic beverages, such as lemon juice, then said predetermined pH may be in the range of 3 to 5, such as in the range of 3 to 4, for example approx. pH 3.5.

[0241] The predetermined conductivity may for example be a conductivity in the range of 3 to 5, preferably in the range of 3 to 4, such as approx. 3.5.

[0242] It is also preferred that the pH at no time during the method exceeds pH 4, more preferably that the pH at no time during the method exceed pH 4.5, yet more preferably that the pH at no time during the method exceed pH 5. In one embodiment of the disclosure, the pH does at no time during the method exceed pH 3.5. This may in particular be the case, when the starting liquid is a fruit or berry juice or extract.

[0243] It is also preferred that the pH at no time during the process is significantly higher than said predetermined pH. Thus, preferably the pH is not more than 20% higher, preferably not more than 15% higher than the predetermined pH at any time during the process. This may for example be ensured by running the CX-REED in parallel with the AX-REED.

[0244] The term "approx." is used herein to indicate +/-10%, preferably +/-5%.

Aroma compound

[0245] It is an advantage of the methods of the disclosure that generally one or more aroma compounds present in the starting liquid are retained in the AX-REED liquid, in the CX-REED liquid, the REED liquid and the final beverages prepared by the methods. Thus, the REED liquid retain aroma compound(s) of the starting liquid, and thus also the final beverage retains aroma compound(s) of the starting liquid. Which aroma compound present in the final beverage of course depends on the aroma compounds present in the starting liquid. An advantage of the present disclosure is that the final beverage may retain has a taste similar to the starting liquid, but with a reduced acid and/or sugar content.

[0246] In embodiments of the disclosure not comprising a step c), then the final beverage will in general be similar to starting liquid except for being less acidic. As described above, the AX-REED liquid, the CX-REED liquid, the REED liquid and the final beverages prepared by the methods of the disclosure retains at least one micro-nutrient and preferable several micro-nutrient. In addition, the AX-REED liquid, in the CX-REED liquid, the REED liquid and/or the final beverages also retains aroma compounds.

[0247] Thus, in one embodiment the disclosure relates to a method of producing a beverage comprising the steps of:

a) providing a starting liquid comprising at least one micro-nutrient, at least one aroma compound, and at least one organic acid; and

d) removing at least 10% of at least one organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient and at least 65% of said at least one aroma compound in said liquid, thereby obtaining an AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0248] The disclosure also relates to a method of producing a beverage comprising the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient, at least one aroma compound and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose; and
3. c) incubating said liquid with one or more glucose fermenting microorganisms capable of fermenting glucose to an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient and at least 65% of said at least one aroma compound in said liquid, thereby obtaining an AX-REED liquid wherein said organic acid

is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0249] In addition the disclosure relates to a method for producing a beverage, wherein the method comprises the steps of

1. a) providing a starting liquid comprising at least one micro-nutrient, at least one aroma compound and at least one sugar; and
2. b) if said sugar is not glucose converting at least some of said sugar to glucose for example by contacting the starting liquid with an enzyme capable of catalysing conversion the particular sugar in question to glucose.; and
3. c) incubating said liquid with an enzyme or a mixture of enzymes capable of catalysing conversion of glucose to form an organic acid; and
4. d) removing at least 10% of said organic acid from said liquid while retaining at least 65% of said at least one micro-nutrient and at least 65% of said at least one aroma compound in said liquid, thereby obtaining an AX-REED liquid wherein said organic acid is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0250] All of the methods described herein above in this section "Aroma compounds" may furthermore contain a step e)

e) removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient and at least 65% of said at least one aroma compound in said liquid, thereby obtaining a CX-REED liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0251] All of the methods described herein above in this section "Aroma compounds" may correspond to any of the methods described herein above in the sections "Method of producing a beverage", "Methods of producing a fermented beverage", "Methods of producing a beverage with enzymatic conversion of sugar" and "Method of producing a beverage without bacterial fermentation" except that the starting liquid furthermore comprises at least one aroma compound and that at least 65% of said aroma compound is retained during both AX-REED and CX-REED treatment. Thus, in relation to f the methods described herein above in this section "Aroma compounds", then step a), b), c), d) and e) may be performed in any of the ways described herein above in the sections "Method of producing a beverage", "Methods of producing a fermented beverage", "Methods of producing a beverage with enzymatic conversion of sugar" and "Method of producing a beverage without bacterial fermentation" with the only difference being that the starting liquid must comprise at least one aroma compound and that at least 65% of said aroma compound is retained in the AX-REED liquid and/or in the CX-REED liquid and/or in the REED liquid.

[0252] Thus the methods described herein above in this section "Aroma compounds" may also contain one or more of the steps f), g), h) and i) as described herein in the sections "Method of producing a beverage", "Methods of producing a fermented beverage", "Methods of producing a beverage with enzymatic conversion of sugar" and "Method of producing a beverage without bacterial fermentation".

[0253] Step d) may comprise removing at least 10% of one or more acidic ions from said liquid while retaining at least 65% of at least one aroma compound in said liquid. In particular step d) may comprise removing at least 10% of one or more acidic ions from said liquid while retaining at least 80% of at least one aroma compound in said liquid.

[0254] Thus step d) may comprise removing at least 10% of one or more acidic ions from said liquid while retaining at least 90% of at least one aroma compound in said liquid. For example step d) may comprise removing at least 10% of one or more acidic ions from said liquid while retaining at least 65% of at least two, such as at least three different aroma compounds in said liquid. Thus step d) may comprise removing at least 10% of one or more acidic ions from said liquid while retaining at least 80% of at least two, such as of at least three different micro-nutrients in said liquid.

[0255] The methods of the disclosure may also comprise a step e) removing at least part of one cation from the AX-REED liquid or from the starting liquid or from the partly AX-REED treated liquid, while retaining at least 65% of at least one aroma compound in said liquid. In particular step e) may comprise removing at least part of one cation from the AX-REED liquid, the partly AX-REED treated liquid or the starting liquid, while retaining at least 80% of at least one aroma compound in said liquid. Thus step e) may comprise removing at least part of one cation from the AX-REED liquid, the partly treated AX-REED liquid or the starting liquid, while retaining at least 90% of at least one aroma compound in said liquid.

[0256] For example step e) may comprise removing at least part of one cation from the AX-REED liquid, the partly AX-REED treated liquid or the starting liquid, while retaining at least 65% of at least two, such as at least three different aroma compound in said liquid. Thus step e) may comprise removing at least part of one cation from the AX-REED liquid, the starting liquid or the partly AX-REED treated liquid while retaining at least 80% of at least two, such as of at least three different aroma compounds in said liquid.

[0257] In certain embodiments of the disclosure, steps d) and e) are performed at least partly simultaneously. The resulting liquid may in these embodiments be referred to as the "REED liquid". It is preferred that the REED liquid retains at least 65% of at least one aroma compound present in the starting liquid.

[0258] In particular steps d) and e) may together comprise removing at least 10% of one or more acidic ions and at least part of one cation from the starting liquid while retaining at least 80% of at least one aroma compound in the REED liquid.

[0259] Thus steps d) and e) may together comprise removing at least 10% of one or more acidic ions and at least part of one cation from the starting liquid while retaining at least 90% of at least one aroma compound in the REED liquid.

[0260] For example steps d) and e) may together comprise removing at least 10% of one or more acidic ions and at least part of one cation from the starting liquid, while retaining at least 65% of at least two, such as at least three different aroma compounds in the REED liquid.

[0261] Thus steps d) and e) may together comprise removing at least 10% of one or more acidic ions and at least part of one cation from the starting liquid while retaining at least 80% of at least two, such as of at least three different aroma compounds in the REED liquid.

[0262] The aroma compound may be any aroma compound, which is desirable to maintain in the final beverage. In general, the aroma compound is a compound with significantly contributes to the taste characteristics of the starting liquid. The skilled person will know, which aroma compounds contribute to the taste characteristics of a given starting liquid.

[0263] The aroma compound is a chemical compound that has a smell or odor. A chemical compound has a smell or odor when it is sufficiently volatile to be transported to the olfactory system in the upper part of the nose. Generally the aroma compound is an organic compound with a molecular weight of less than 300.

[0264] The aroma compound may for example be an ester. Non-limiting examples of aroma compounds, which are esters include geranyl acetate, methyl formate, methyl propionate, methyl propanoate, methyl butyrate, methyl butanoate, ethyl butyrate, ethyl butanoate, isoamyl acetate, pentyl butyrate, pentyl butanoate, pentyl pentanoate, octyl acetate, benzyl acetate, ethyl anthranilate, fructose, hexyl acetate, ethyl methylphenylglycidate,

[0265] The aroma compound may also be a terpene or a terpenoid. Terpenes are molecules consisting of covalently linked units of isoprene, and thus terpenes have the molecular formula $(C_5H_8)_n$, where n is the number of linked isoprene units. Terpenoids are terpenes, which are modified chemically, such as by oxidation or rearrangement of the carbon skeleton.

[0266] Non-limiting examples of aroma compounds, which are linear terpenes or terpenoids include geraniol, nerol, citral, lemonal, geranial, neral, citronellal, citronellol or linalool. Non-limiting examples of aroma compounds, which are cyclic terpenes or terpenoids include limonene or thujone.

[0267] The aroma compound may also for example be an aromatic compound. In particular the aroma compound may be an aromatic compound consisting of a 6 membered aromatic ring substituted with one or more substituents. Non-limiting examples of aroma compounds, which are aromatic compounds include benzaldehyde, cinnamaldehyde, ethyl maltol, vanillin, anisole, anethole, estragole and thymol.

[0268] Other non-limiting examples of aroma compounds include furaneol, 1-hexanol, menthol, isovaleraldehyde, anisic aldehyde, dihydrojasnone, gamma-decalactone, gamma-nonolactone, delta-octalactone, jasmine lactone, massoia lactone, wine lactone, sotolon, grapefruit mercaptan, furan-2-yl methanethiol, phosphine or nerolin.

Organic acid

[0269] As used herein the term "organic acid" refers to any carboxylic acid. Preferably, the organic acid according to the disclosure is C₁₋₃-alkyl or C₁₋₃-alkenyl, wherein said C₁₋₃-alkyl and C₁₋₃-alkenyl is substituted with n -COOH groups, m -OH group and q =O groups, wherein n is an integer in the range of 1 to 3, m is an integer in the range of 0 to 2 and q is an integer in the range of 0 to 1.

[0270] Preferably the organic acid may be propyl substituted with

1. 1) 1 to 3 -COOH groups, such as with 3 -COOH groups; and
2. 2) 0 to 1 -OH groups, such as with 1 -OH group

or

the organic acid may be ethyl substituted with

1. 1) 1 to 2 -COOH groups; and
2. 2) 0 to 2 -OH groups.

[0271] Preferably the term "organic acid" as used herein refers to lactic acid, citric acid, malic acid, tartaric acid, acetic acid, succinic acid, isocitric acid, α-ketoglutaric acid, fumaric acid and oxaloacetic acid.

[0272] In one very preferred embodiment of the disclosure the term "organic acid" as used herein refers to lactic acid.

Sugar

[0273] As used herein the term "sugar" refers collectively to monosaccharides, disaccharides and tri-saccharides.

[0274] Monosaccharides in general have the chemical formula C_x(H₂O)_y, where x is 3 to 7. Thus the monosaccharide according to the disclosure may be selected from the group consisting of triose, tetrose, pentose, hexose and heptose. Preferably the monosaccharide is a hexose.

[0275] Disaccharides are di-mers of monosaccharides and tri-saccharides are tri-mers of monosaccharides.

[0276] Preferably, the term "sugar" as used herein refers to fructose, maltose, maltotriose, lactose, sucrose and glucose.

Ratio of sugar to organic acid

[0277] Interestingly, the inventors have found that certain ratios of sugar to organic acids provide beverages, which are particularly palatable.

[0278] Accordingly it is preferred that the beverages prepared by the methods of the present disclosure have a ratio of sugar to organic acid in the range of 1:2 to 60:1. In particular, the ratio of sugar to organic acid may be in the range of 5.5:1 to 10:1, more preferably in the range of 6:1 to 10:1, such as in the range of 7:1 to 9:1, for example in the range of 8:1 to 9:1. The ratio is calculated as total concentration of sugar in g/L to total concentration of organic acids in g/L.

[0279] Frequently, a beverage may be palatable when it comprises a minimum amount of sugar, whereas adding more sugar does not improve the taste. Thus, in general it is preferred that the beverages of the disclosure comprises as little sugar as possible, but still being palatable. Thus, the ratios given herein in general refer to beverages containing as low a sugar content as possible, but still being perceived palatable.

[0280] For beverages prepared by methods including step c), then the ratio of sugar to organic acid in the beverages may be in the range of 20:1 to 60:1, for example in the range of 25:1 to 55:1. However, it is preferred that the ratio of sugar to organic acid are in the range of 5.5:1 to 10:1, more preferably in the range of 6:1 to 10:1, such as in the range of 7:1 to 9:1, for example in the range of 8:1 to 9:1. This is in particular the case when the beverages are prepared from a starting liquid comprising a malt extract and/or wort.

[0281] For beverage prepared by methods not including step c), then it is preferred that the ratio of sugar to organic acid is in the range of 1:2 to 10:1, such as in the range of 1:1.5 to 5:1, for example in the range of 1.2:1 to 1.1.2. This is in particular the case when the beverages are prepared from a starting liquid comprising fruit juice or a fruit extract, for example a citrus fruit juice.

[0282] In particular, the ratio of sugar to organic acids may be the ratio of

1. I. the total concentration in g/L of monosaccharides and disaccharides; to
2. II. the total concentration in g/L of organic acids, which are C₁₋₃-alkyl or C₁₋₃-alkenyl, wherein said C₁₋₃-alkyl and C₁₋₃-alkenyl is substituted with n - COOH groups, m -OH

group and $q = \text{O}$ groups, wherein n is an integer in the range of 1 to 3, m is an integer in the range of 0 to 2 and q is an integer in the range of 0 to 1.

[0283] For example the ratio of sugar to organic acid may be the ratio of

1. I. the total concentration in g/L of fructose, maltose, lactose, sucrose and glucose; to
2. II. the total concentration in g/L of lactic acid, citric acid, malic acid, tartaric acid, acetic acid, succinic acid, isocitric acid, α -ketoglutaric acid, fumaric acid and oxaloacetic acid.

[0284] In one embodiment of the disclosure the ratio of sugar to organic acid may be the ratio of

1. I. the total concentration in g/L of glucose; to
2. II. the total concentration in g/L of lactic acid..

[0285] In these embodiments of the disclosure it is preferred that the ratio of glucose to lactic acid is in the range of 5:1 to 10:1, more preferably in the range of 6:1 to 9:1, such as in the range of 7:1 to 8:1. This is in particular the case for methods of the disclosure comprising a step c) and/or for methods of the disclosure wherein the starting liquid comprises a malt extract and/or wort.

Micro-nutrient

[0286] The term "micro-nutrient" as used herein refers to nutrients required by humans in small quantities, which the organism itself cannot produce. Thus the term micro-nutrient is not meant to include sugars, proteins and fats, or other calorie containing nutrient.

[0287] The micro-nutrient according to the invention is selected from the group consisting of minerals, vitamins, salts and anti-oxidants.

[0288] The methods of the disclosure comprises a step d) of removing at least 10% of one or more acidic ions from the liquid, while retaining at least some of a micro-nutrient. In relation to any of the methods of producing a beverage described herein, and in particular in relation to any of the methods described in the section "Method of producing fermented beverage", and in relation to any of the methods described in the section "Method of producing beverages with enzymatic conversion of sugar" and in relation to any of the methods described in the section "Method of producing a beverage without bacterial fermentation" step d) may be as follows:
Step d) may comprise removing at least 10% of one or more acidic ions from said liquid while

retaining at least 65% of at least one micro-nutrient in said liquid, wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0289] In particular step d) may comprise removing at least 10% of one or more acidic ions from said liquid while retaining at least 80% of at least one micro-nutrient in said liquid, wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0290] Thus step d) may comprise removing at least 10% of one or more acidic ions from said liquid while retaining at least 90% of at least one micro-nutrient in said liquid, wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0291] For example step d) may comprise removing at least 10% of one or more acidic ions from said liquid while retaining at least 65% of at least two, such as at least three different micro-nutrients in said liquid, wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0292] Thus step d) may comprise removing at least 10% of one or more acidic ions from said liquid while retaining at least 80% of at least two, such as of at least three different micro-nutrients in said liquid, wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack.

[0293] The methods of the disclosure may also comprise a step e) removing at least part of one cation from the AX-REED liquid, while retaining at least some of a micro-nutrient. In relation to any of the methods of producing a beverage described herein, and in particular in relation to any of the methods described in the section "Method of producing fermented beverage", and in relation to any of the methods described in the section "Method of producing beverages with enzymatic conversion of sugar" and in relation to any of the methods described in the section "Method of producing a beverage without bacterial fermentation" step e) may be as follows:

Step e) may comprise removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of at least one micro-nutrient in said liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0294] In particular step e) may comprise removing at least part of one cation from the AX REED liquid while retaining at least 80% of at least one micro-nutrient in said liquid, wherein said cation is removed through an Cation Exchange Reverse Electro Enhanced Dialysis (CX-REED) membrane stack.

[0295] Thus step e) may comprise removing at least part of one cation from the AX-REED

liquid while retaining at least 90% of at least one micro-nutrient in said liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0296] For example step e) may comprise removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of at least two, such as at least three different micro-nutrients in said liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack.

[0297] Thus step e) may comprise removing at least part of one cation from the AX-REED liquid while retaining at least 80% of at least two, such as of at least three different micro-nutrients in said liquid, wherein said cation is removed through an Cation Exchange Reverse Electro Enhanced Dialysis (CX-REED) membrane stack

[0298] In certain embodiments of the disclosure, steps d) and e) are performed at least partly simultaneously. The resulting liquid may in these embodiments be referred to as the "REED liquid". It is preferred that the REED liquid retains at least 65% of at least one micro-nutrient present in the starting liquid.

[0299] In particular steps d) and e) may together comprise removing at least 10% of one or more acidic ions and at least part of one cation from the starting liquid while retaining at least 80% of at least one micro-nutrient in the REED liquid.

[0300] Thus steps d) and e) may together comprise removing at least 10% of one or more acidic ions and at least part of one cation from the starting liquid while retaining at least 90% of at least one micro-nutrient in the REED liquid.

[0301] For example steps d) and e) may together comprise removing at least 10% of one or more acidic ions and at least part of one cation from the starting liquid, while retaining at least 65% of at least two, such as at least three different micro-nutrients in REED liquid.

[0302] Thus steps d) and e) may together comprise removing at least 10% of one or more acidic ions and at least part of one cation from the starting liquid while retaining at least 80% of at least two, such as of at least three different micro-nutrients in the REED liquid.

[0303] When the micro-nutrient is a salt, it may for example be phosphate or iodide.

[0304] When the micro-nutrient is a mineral it may for example be selected from the group consisting of potassium, iron, calcium, cobalt, chromium, copper, manganese, magnesium, selenium, zinc, molybdenum and silicon. In embodiments of the disclosure when the micro-nutrient is a mineral, it is preferred that said mineral is a mineral, the intake of which is beneficial to human health. Accordingly, it is preferred that the micro-nutrient is a mineral

selected from the group consisting of calcium, magnesium, iron and silica. In some embodiments of the disclosure it is preferred that the mineral is selected from the group consisting of calcium, magnesium and iron.

[0305] When the starting liquid comprises a malt extract and/or wort, then the micro-nutrient may in particular be calcium, magnesium, potassium, silicon, iron or zinc, more preferably the micro-nutrient may be calcium, magnesium or iron. Thus it is preferred that at least one of, more preferably at least 2 of, even more preferably at least 3 of, yet more preferably all of calcium, magnesium and iron are retained in the liquid during removal of acidic ions and in embodiments of the disclosure containing a step e) also during removal of a cation.

[0306] The micro-nutrients may also be vitamins. When the micro-nutrient is a vitamin said vitamin may preferably be selected from the group consisting of vitamin A, vitamin B₁, vitamin B₂, vitamin B₃, vitamin B₆, vitamin B₉, vitamin E and vitamin K. In particular, the micro-nutrient may be selected from the group consisting of vitamin B₆ and vitamin B₁₂.

[0307] When the starting liquid comprises a malt extract and/or wort then the micro-nutrient may in particular be vitamin A, vitamin B₁, vitamin B₂, vitamin B₃, vitamin B₆, vitamin B₉, vitamin E or vitamin K. Thus it is preferred that at least one of, more preferably at least 2 of, even more preferably at least 3 of, yet more preferably all of vitamin A, vitamin B₁, vitamin B₂, vitamin B₃, vitamin B₆, vitamin B₉, vitamin E or vitamin K are retained in the liquid during removal of acidic ions and in embodiments of the disclosure containing a step e) also during removal of a cation.

[0308] In embodiments of the disclosure involving a bacterial fermentation, then the micro-nutrient is preferably not a vitamin. In said embodiments it is preferred that the micro-nutrient is a mineral, such as any of the above-mentioned minerals.

[0309] In embodiments of the disclosure, wherein the starting liquid comprises a malt extract and/or wort and wherein the methods do not involve a bacterial fermentation, e.g. in any of the methods described herein above in the sections "Method of producing beverages with enzymatic conversion of sugar" and "Methods of producing a beverage without bacterial fermentation" then the micronutrient may for example be selected from the group consisting of minerals and vitamins, Said minerals may for example be any of the minerals described herein above, The vitamins may also be any of the vitamins described herein above, but preferably said vitamins are selected from the group consisting of vitamin B₁ and vitamin B₂. Thus, in these embodiments it is preferred that at least 65% of the vitamin B₁ is retained in the liquid after step d). It is furthermore preferred that at least 65% the vitamin B₁ is retained in the liquid after step e). Thus, it is preferred that the final beverage comprises at least 65% of the vitamin B₁ present in the starting liquid. In addition, in these embodiments it is preferred that at least 65% of the vitamin B₂ is retained in the liquid after step d). It is furthermore preferred that at least 65% the vitamin B₂ is retained in the liquid after step e). Thus, it is preferred that the final beverage comprises at least 65% of the vitamin B₂ present in the starting liquid.

[0310] In embodiments of the disclosure, wherein the starting liquid comprises a malt extract and/or wort and wherein the methods do not involve a bacterial fermentation, e.g. in any of the methods described herein above in the sections "Method of producing beverages with enzymatic conversion of sugar" and "Methods of producing a beverage without bacterial fermentation" then it is preferred that at least 65% of at least 2, more preferably of at least 3, yet more preferably of all micro-nutrients selected from the group consisting of calcium, magnesium, iron, vitamin B₁ and vitamin B₂ are retained in the liquid during removal of acidic ions and in embodiments of the disclosure containing a step e) also during removal of a cation.

[0311] The micro-nutrient may also be antioxidants, such as polyphenols. Said polyphenol may for example be a flavonoid, such as quercetin or catechin or an anthocyanin.

[0312] In one embodiment of the disclosure one micro-nutrient is the total level of antioxidants determined using the oxygen radical absorbance capacity (ORAC) method. Thus, it is preferred that the final beverage comprises at least 65% of the total level of antioxidants contained in the starting liquid, wherein the total level of antioxidants is determined by the ORAC method. In particular, the total level of antioxidants may be determined using ORAC-FL, preferably as described in Dávalos et al., 2004, J.Agric.Food Chem., 52, p. 48-54.

[0313] When the starting liquid comprises or consists of a fruit juice and the methods do not involve a step of fermentation, then it is preferred that the micro-nutrient may in particular be a mineral, a vitamin and/or an antioxidant. The mineral may then in particular be calcium, magnesium, silica and/or iron. Thus it is preferred that at least one of, more preferably at least 2 of, even more preferably at least 3 of, yet more preferably all of calcium, magnesium, silica and iron are retained in the liquid during removal of acidic ions and in embodiments of the disclosure containing a step e) also during removal of a cation. In these embodiments it is also preferred that the final beverage comprises at least 65% of the total level of antioxidants contained in the starting liquid.

[0314] The methods according to the disclosure comprise removal of acidic ions and optionally cations, while at least one micro-nutrient is retained in the liquid and thus present in the final beverage. The term "retaining said at least one micro-nutrient" as used herein means that the concentration of said at least one micro-nutrient has not decreased by more than 10%, for example the concentration of said micro-nutrient has not decreased by more than 5% during performing step d) or in embodiments of the disclosure comprising step e) then during performing steps d) and e). Even more preferably "retaining said at least one micro-nutrient" means that the concentration of said micro-nutrient is the same or higher after performance of step d) compared to the level of said micro-nutrient in the starting liquid. In embodiments of the disclosure comprising step e) then it is preferred that the term "retaining said at least one micro-nutrient" means that the concentration of said micro-nutrient is the same or higher after performance of steps d) and e) compared to the level of said micro-nutrient in the starting liquid.

[0315] It is preferred that the level of at least one micro-nutrient, preferably of at least two micro-nutrients, even more preferably of at least 3 micro-nutrients, such as of at least 4 micro-nutrients, for example of at least 5 micro-nutrients, such as of at least 6 micro-nutrients is retained during the methods of the disclosure.

[0316] More preferably the level of at least one micro-nutrient, preferably of at least two micro-nutrients, even more preferably of at least 3 micro-nutrients is retained during the methods of the disclosure, wherein said micro-nutrients are selected from the group consisting of phosphate, iodine, potassium, iron, calcium, cobalt, chromium, copper, manganese, magnesium, selenium, zinc, molybdenum, silicon, vitamin A, vitamin B₁, vitamin B₂, vitamin B₆, vitamin E and vitamin K .

[0317] In one preferred embodiment of the disclosure the level of at least one micro-nutrient, preferably of at least two micro-nutrients, even more preferably of at least 3 micro-nutrients are retained during the methods of the disclosure, wherein said micro-nutrients are selected from the group consisting of calcium, magnesium, potassium, vitamin B₆ and vitamin B₁₂.

[0318] In one preferred embodiment of the disclosure, the starting liquid comprises a malt extract and/or wort and the level of at least one micro-nutrient, preferably of at least two micro-nutrients, even more preferably of at least 3 micro-nutrients are retained during the methods of the disclosure, wherein said micro-nutrients are selected from the group consisting of calcium, magnesium, potassium, vitamin B₆ and vitamin B₁₂.

[0319] In embodiments of the disclosure wherein the starting liquid comprises a high level of vitamin C, then one micronutrient may be vitamin C. It is preferred that at least 40%, such as at least 45% of the vitamin C of the starting liquid is retained in the final beverage. Thus, in embodiments of the disclosure where the starting liquid comprises at least 100 mg/L, such as at least 200 mg/L, for example at least 300 mg/L, then it is preferred that at least at least 40%, such as at least 45% of the vitamin C of the starting liquid is retained in the CX-REED liquid or in the REED liquid.

AX-REED

[0320] The methods of the disclosure contains a step of removing one or more acidic ions from the liquid while retaining at least some of said at least one micro-nutrient in said liquid (e.g. retaining at least 65% of said at least one micro-nutrient in said liquid), wherein said acidic ion is removed through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack. This step is designated step d) herein above. As used herein the term "removing an organic acid" refers to removing the acidic ion of said organic acid.

[0321] Step d) involves removing one or more acidic ions from said liquid while retaining at least 65% of said at least one micro-nutrient in said liquid, wherein said acidic ion is removed

through an Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED) membrane stack, said membrane stack containing

1. i) at least one cell consisting of:
 1. 1. two an-ion exchange membranes defining a chamber for the starting liquid; and
 2. 2. two further chambers for a dialysis liquid, wherein said two further chambers are positioned adjacent to the chamber for the starting liquid on opposing sides and wherein said two further chambers may be connected
2. ii) a set of end membranes
3. iii) means for applying an electric field over the membrane stack by means of at least two electrodes
4. iv) means for reversing the direction of the electric field within said membrane stack

and wherein the removal involves the steps of

1. i) inserting the starting liquid into the chamber for the starting liquid; and
2. ii) inserting a dialysis liquid in the two further chambers for the dialysis liquid; and
3. iii) applying an electric field over the membrane stack;
4. iv) incubating said starting liquid in said chamber, whereby the direction of electric field is reversed at intervals

[0322] In general step d) involves removing at least 10%, for example at least 15%, for example at least 20%, such as at least 25%, such as at least 30% of one or more acidic ions.

[0323] This step may also be referred to as AX-REED treatment. The liquid obtained after AX-REED treatment may be referred to as "AX-REED" liquid herein. In certain embodiments of the disclosure, then step d) is the last step of the method, and in these embodiments the liquid obtained after AX-REED treatment is the beverage. In other embodiments of the disclosure the method furthermore involves a step e) of removing at least part of one cation from the liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack. This may also be referred to as CX-REED treatment. Steps d) and e) may collectively be referred to as "REED treatment" herein.

[0324] Frequently steps d) and e) may be performed using only one apparatus, which may be referred to as "REED equipment" herein. REED equipment to be used with the present disclosure may in general be set up to perform step d) and step e) in separate compartments. The term "REED equipment" as used herein is an apparatus comprises at least one AX-REED membrane stack. Preferably a REED equipment according to the present disclosure comprises at least one AX-REED membrane stack and at least one CX-REED membrane stack.

[0325] Thus, steps d) and e) may be performed simultaneously or sequentially, and preferably both steps d) and e) are performed using REED equipment set up to perform both steps d)

and e). An overview of suitable REED equipment is shown in figure 1 and in fig. 4 which illustrates a REED setup which may be preferred in several embodiments of the present disclosure. It is however possible to perform steps d) and e) using separate REED equipment, which then may be referred to as AX-REED equipment and CX-REED equipment, respectively.

[0326] Thus, the REED equipment according to the invention comprises at least one AX-REED membrane stack and at least one CX-REED membrane stack, which may be any of the AX-REED membrane stacks described herein below in this section and any of the CX-REED membrane stacks described herein below in the section CX-REED. According to the invention, the REED equipment contains at least one AX-REED and at least one CX-REED membrane stack, wherein said AX-REED and said CX-REED membrane stacks are connected in parallel. Thus, the REED equipment may contain one AX-REED membrane stack and one CX-REED membrane stack connected in parallel.

[0327] When two or more REED stacks are arranged in parallel the treated fluid i.e. the starting liquid from one REED stack is not lead directly to the next REED stack as would be the case if the two stack were connected in series.

[0328] A parallel system according to the invention has an AX-REED and a CX-REED connected to a reservoir and/or tank with a starting liquid. The AX-REED receives starting liquid from the reservoir and/or tank and said starting liquid is returned to the reservoir and/or system after being treated in the AX-REED stack. Simultaneously or at another time the CX-REED receives starting liquid or partly AX-REED treated liquid or AX-REED liquid from the reservoir and/or tank and said liquid is returned to the reservoir and/or tank after being treated in the CX-REED stack. It is understood that the liquid may be recirculated to the AX-REED and/or CX-REED stack from the tank. Such re-circulated liquid will in principle be partly AX-REED and/or CX-REED treated liquid. For the sake of simplicity, the chambers of the AX-REED and CX-REED membrane stack may be referred to as "chamber for starting liquid" even if also partly AX-REED and/or CX-REED treated liquid may be introduced into these chambers.

[0329] The REED equipment may alternatively comprise more AX-REED membrane stacks than CX-REED Stacks or the REED equipment may comprise more CX-REED membrane stacks than AX-REED Stacks. The relative number of AX-REED membrane stacks/ CX-REED membrane stacks than can be varied in order to regulate how much of a first component is removed from the liquid relatively to how much of a second component is removed from the liquid. The ratio between removed first component and removed second component may also be adjusted by providing AX-REED membrane stacks and CX-REED membrane stacks of different sizes.

[0330] A REED stack comprises at least one chamber for starting liquid and at least two chambers for dialysis liquid. The chamber(s) containing starting liquid and the chambers containing dialysis liquid are arranged alternatingly side by side i.e. a REED stack comprises at least three active adjacent chambers: a chamber for dialysis liquid - a chamber for starting liquid - a chamber for dialysis liquid. Each interface between a chamber for starting liquid and

chamber for dialysis liquid is formed by anion exchange membrane, which exchange membranes in the AX-REED stacks are anion exchange membranes and in the CX-REED stacks are cation exchange membranes.

[0331] Each REED stack also comprises two end membranes defining an electrode chamber at each end of the REED stack i.e. a REED stack with two end membranes comprises at least five adjacent chambers: an electrode chamber -a chamber for dialysis liquid - a chamber for starting liquid - a chamber for dialysis liquid - an electrode chamber.

[0332] It is to be understood that in embodiments of the disclosure where step e) is performed after step d), then in so far as the CX-REED membrane stack is concerned then the "chamber for starting liquid" in fact filled with AX-REED liquid. Furthermore, it is to be understood that after running AX-REED or CX-REED for a while, then the liquid in the chamber for starting liquid may be partly REED treated starting liquid. In embodiments of the disclosure where the AX-REED treatment is performed alone for a while followed by simultaneous AX-REED and CX-REED in parallel, the liquid inserted into the "chamber for the starting liquid" of the CX-REED membrane stack, is in fact partly AX-REED treated starting liquid. For the sake of simplicity the chamber is never-the-less referred to as "chamber for starting liquid".

[0333] Each electrode chamber can be formed by an end membrane and an end wall of the REED stack.

[0334] A REED stack with seven adjacent chambers, two electrode chambers and five active chambers are arranged as: an electrode chamber -a chamber for dialysis liquid - a chamber for starting liquid - a chamber for dialysis liquid - a chamber for starting liquid - a chamber for dialysis liquid -an electrode chamber.

[0335] Fig. 4 shows an exemplary REED equipment 1 according to the present disclosure said REED equipment comprises an AX-REED stack 2 arranged in parallel with a CX-REED stack 3. Both AX-REED and CX-REED stack is connected to a tank 4 containing the liquid by piping 5 and to a fluid system 6a and 6b providing and leading dialysis liquids to and away from the REED stacks. The fluid system 6a is for providing the dialysis liquid to be used with AX-REED, whereas the fluid system 6b is for providing the second dialysis liquid. At the beginning of the process the tank 4 contains the starting liquid, later the tank contains the partly AX-REED and/or CX-REED treated liquid. At the end of the process the tank 4 contains either the AX-REED liquid, the CX-REED liquid or the REED liquid.

[0336] The AX-REED stack comprises a first 7 and second 8 electrode arranged to provide an electrical field across five active chambers in between the electrodes i.e. across the alternating chambers with dialysis liquid 9 and starting liquid 10 formed by the membranes. In the present exemplary stack the alternating chambers are formed by:

- an end membrane 11a defining at one side a first electrode chamber 7a and on the opposing side a first chamber 9 for dialysis liquid

- a first anion exchange membrane 12a which together with the first end membrane defines the first chamber,
- Second anion exchange membrane 12b which together with the first anion exchange membrane forms a second chamber 10a for starting liquid
- third anion exchange membrane 12c which together with the second anion exchange membrane forms a third chamber 9b for dialysis liquid
- fourth anion exchange membrane 12d which together with the third anion exchange membrane forms a fourth chamber 10b for dialysis liquid
- a second end membrane 11b which together with the fourth anion exchange membrane forms a fifth chamber 9c for dialysis liquid

The first and second electrode are arranged in a first 7a and second 8a electrode chamber respectively. Said first electrode chamber defined by a first end wall (indicated by dotted line) and the first end membrane and said second electrode chamber defined by second end wall (also indicated by dotted line) and the second end membrane.

[0337] The exchange membranes 12a - 12d can preferably be of the same type as well as the two end membranes also can be identical.

[0338] Similarly the CX-REED stack comprises two electrodes 13 and 14 one on each side of a stack of membranes said stack of membranes being a first end membrane 15a , four cation exchange membranes 16a-16d and a second end membrane 15b. Said membranes together with end walls forming a first electrode chamber 13a, a first chamber for dialysis liquid 17a, a first chamber for starting liquid 18a, a second chamber for dialysis liquid 17b, a second chamber for starting liquid 18b, a third chamber for dialysis liquid 17c and a second electrode chamber 14a.

[0339] In the present example dialysis liquid may be any of the dialysis liquid to be used with AX-REED described in this section, and the second dialysis liquid may be any of the second dialysis liquids described in the section "CX-REED".

[0340] It is also comprised within the present disclosure that steps b), c), d) and e) may be performed simultaneously. This may for example be done within the REED equipment. It is also comprised within the disclosure that steps c), d) and e) are performed simultaneously. This may for example be done within the REED equipment. In particular this may be done using a REED equipment containing one AX-REED membrane stack and one CX-REED membrane stack connected in parallel.

The REED equipment may also contain more than one AX-REED membrane stacks connected in series, wherein said AX-REED membrane stacks are connected in parallel to at least one CX-REED membrane stack. The REED equipment may also contain more than one AX-REED membrane stacks connected in series, and more than one CX-REED membrane stacks connected in series, wherein said AX-membrane stacks and CX-REED membrane stacks are connected to each other in parallel.

[0341] In some embodiments of the disclosure the methods do not comprise steps b) and c), in which case, step d) is generally performed after step a). In other embodiments of the disclosure the methods do not comprise steps b) and c), but comprise a step e), in which case, step d) is generally performed after step a) and prior to step e), wherein steps d) and e) may be repeated p number of times, where p is an integer in the range of 1 to 5.

[0342] Step d) of the methods of the disclosure involves use of one or more AX-REED membrane stacks, wherein each of said membrane stacks contains

v) at least one cell consisting of:

1. 1. two an-ion exchange membranes defining a chamber for the starting liquid; and
2. 2. two further chambers for a dialysis liquid, wherein said two further chambers are positioned adjacent to the chamber for the starting liquid on opposing sides and wherein said two further chambers may be connected

v) a set of end membranes

vi) means for applying an electric field over the membrane stack by means of at least two electrodes

vii) means for reversing the direction of the electric field within said membrane stack. Thus regardless of whether the electric field ions will be able to move from the chamber defining the starting liquid into either of the chambers for the dialysis liquid.

[0343] Each AX-REED membrane stack may comprise more than one cell as identified above. For example each AX-REED membrane stack may comprise in the range of 2 to 100 cells, such as in the range of 2 to 50 cells, such as in the range of 2 to 25 cells.

[0344] The removal of the acidic ion typically involves the steps of

1. i) inserting the starting liquid into the chamber for the starting liquid; and
2. ii) inserting a dialysis liquid in the two further chambers for the dialysis liquid; and
3. iii) applying an electric field over the membrane stack;
4. iv) incubating said starting liquid in said chamber, whereby the direction of electric field is reversed at intervals

[0345] AX-REED may be performed under circulation meaning that after incubation of the starting liquid in said chamber the resulting liquid may be removed from the chamber and later inserted into another chamber for the starting liquid or even into the same chamber for the starting liquid. When inserted into the same chamber, then frequently the dialysis liquid in said two further chambers has been exchanged for a fresh dialysis liquid.

[0346] When more than one membrane stack is employed the starting liquid may be inserted into each of the chambers for the starting liquid separately. Alternatively, some or all of said chambers may be connected, so that some or all of the chambers may be fed simultaneously. Similarly, the dialysis liquid may be inserted into each of the chambers for the dialysis liquid separately. Alternatively, some or all of said chambers may be connected, so that some or all chambers are fed simultaneously.

[0347] The acidic ion to be removed may for example be the anion of any organic acid, for example the anion of any of the organic acids, described herein above in the section "Organic acid".

[0348] During removal of said acidic ion, the two membranes surrounding the chamber for the starting liquid either facilitates the transport of ions out of the starting liquid or into the starting liquid from the dialysis liquid.

[0349] The direction of the electric field is changed at intervals. Each reversal of the direction of the electric field results in a short-term reestablishment of the affected ions' polarization profiles at the surface and inside the membranes, as the two membranes surrounding each feed compartment exchanges functions. This causes a short-term reversal of the separation process as the ions previously being removed are pushed back into the feed solution until the membrane profiles are re-established. It is advantageous to keep the intervals between current reversals within any one REED stack as long as allowed by the build-up of fouling, as each reversal introduces a short separation pause and introduces minor process instability.

[0350] The methods of the disclosure may involve use of more than one AX-REED membrane stack. The membrane stacks may be stacked (commonly separated by membrane spacers) on top of each other or side by side until sufficient membrane separation area is achieved. For feasible handling, operational, and maintenance purposes, the membrane stacks may be operated in several separate, practically sized membrane stacks, each with its own set of flow connections and electrodes, but with the same separation function. These stacks are operated together in parallel or serial or some combination thereof as part of the same separation system. It is advantageous to operate with multiple AX-REED membrane stacks, when more than one set of electrodes are used. The number of AX-REED membrane stacks may thus vary from 2 to several hundred depending on the process in question, but are typically in the range 2-50 AX-REED membrane stacks, more typically in the range of 4-20 membrane stacks.

[0351] The dialysis liquid to be used with AX-REED according to the disclosure may be any alkaline solution. Typically it is an aqueous solution of a cation-OH, wherein said cation typically may be the cation of a metal. For example the dialysis liquid may comprise one or more bases selected from the group consisting of Ca(OH)_2 , Mg(OH)_2 , KOH, and NaOH, preferably from the group consisting of Ca(OH)_2 , Mg(OH)_2 and KOH, The dialysis liquid will typically contain said base in a concentration of in the range of 5 to 80%, preferably in the range of 10 to 70%, more preferably in the range of 20 to 60%, for example in the range of 30 to 50%. In certain embodiments of the disclosure said base is used in a concentration of in the range of 5 to 20%.

This may in particular be the case, when the dialysis liquid is used only once. All percentages are provided as w/w.

[0352] In the case of AX-REED, acidic ions are extracted through one anion-exchange membrane in each cell of the AX-REED membrane stack, while typically hydroxide ions enter through the opposite anion- exchange membrane. When the direction of the electrical field is reversed, the extracted acidic ions inside the first mentioned membrane is pushed back into the starting liquid, before hydroxide ions starts entering the starting liquid. Thus, in the short time period until the hydroxide profile is re-established through the membrane, which was previously used to extract acidic ions, no pH control is observed. The length of the time phase after each current reversal until pH control is regenerated depends on various process conditions and membrane properties; typically, it takes between 10-90 seconds before the process is again operating at optimal process parameter control. This is registered as a sudden change in the process parameter e.g. pH, which must then be regulated back to the desired set-point. In order to spread out the instability effects and reduce the overall impact of current reversals with more than one membrane stack, the reversals of the electric field on each separate stack are preferably performed asynchronously. Thus, it is preferred with the disclosure that more than one AX-REED membrane stack is employed and that the electric field on each separate stack is reversed asynchronously. Even though the intervals for reversing the electrical field of each stack are typically of similar length, the timing of the reversals are dispersed for best process stability effect.

[0353] In an embodiment of the disclosure the direction of the electric field within any first membrane stack is reversed at substantially regular dispersal intervals relative to the reversals for any second or further membrane stack.

[0354] The interval length between current reversals for a stack is typically chosen with regard to the build-up of membrane fouling. Typically, said intervals within any one REED stack may be in the range 5-6000 seconds, preferably 8-3000 seconds, more preferably 10-2000 seconds and even more preferably 100-1500 seconds.

In another embodiment of the disclosure the direction of the electric field within any first membrane stack is reversed at dispersal intervals of substantially even length relative to the reversals for any second or further membrane stack in order to maximise the time between a current reversal of any first REED stack and any second or further REED stack in the same process. With the same dispersal interval length between current reversals, i.e. where these reversals are dispersed evenly the connected bioreactor will experience a reduced impact, but much more often.

[0355] In an embodiment of the disclosure the intensity of the applied electric field is adjusted in response to the pH, target ion concentration or conductivity of said liquid composition. By increasing the intensity of the electric field, the ion exchange increases in the REED system, and vice versa. Online, semi-online (e.g. time-delayed) or secondary (e.g. using online conductivity or turbidity measurements for estimating target ion concentration) measurements of the process parameters being regulated are input in a control regulation mechanism e.g.

PID-control software, which in turn regulates the output of the power supplies to the REED electrodes.

[0356] The current reversal is not the only effect, which can introduce deviations in process control. For optimal control of process parameters, it may be advantageous to control the concentration of various ions in the dialysis liquid as well as the flow and temperature and mode of operation. With regard to temperature, in embodiments of the disclosure where steps c) and d) are performed simultaneously, then the temperature is typically selected in order to allow growth of said glucose fermenting microorganism.

[0357] If multiple stacks are used it is possible to set up the flow of the dialysis liquid either in parallel, or in serial mode with or without booster pumps between stacks, in a similar fashion as with the starting liquid.

[0358] In embodiments of the disclosure where the methods comprise step c), then Anion-Exchange REED (AX- REED) in general serves to replace produced organic acids with hydroxide ions, and thus, countering the reduction of pH from the acid-formation. By regulation of the AX-REED, the hydroxide exchange can maintain pH during fermentation without the need for neutraliser addition.

[0359] In embodiments of the disclosure wherein the methods do not comprise step c), then the AX-REED in general serves to replace organic acids already present in the starting liquid with hydroxide ions and thus increasing the pH of the starting solution.

[0360] In the context of this disclosure the term "reversal of the electric field" or "current reversal" means the changing of polarity of the REED electrodes, resulting in a reversal of the direction of the electrical DC current, which facilitates the migration of ions through the ion-exchange membranes.

[0361] The anion exchange membranes may be any useful anion exchange membranes. The size of the membranes may be selected in order to achieve a suitable retention time. For calculating the retention time, the total area of the anion membranes used is of interest. Accordingly, if the method employs use of many membrane stacks and/or if each membrane stack contains many cells, then the area of each membrane may be reduced.

[0362] Non-limiting examples of useful anion exchange membranes include Ionic AR103 (GE, USA), Neosepta ASM (Astom Corp., Japan), Fumatech FAB (anion)(Fumatech, Germany) or Nafion N117 (anion)(Dupont).

[0363] Non-limiting examples of useful methods and equipment for performing AX-REED are also described in European patent applications EP 1 347 823, EP2349541 and EP2349540.

CX-REED

[0364] In some embodiments, the methods of the disclosure furthermore comprises step e), wherein step e) comprises removing at least part of one cation from the AX-REED liquid, while retaining at least 65% of said at least one micro-nutrient in said liquid, wherein said cation is removed through an Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED) membrane stack, said membrane stack containing

i) at least one cell consisting of:

1. a. two cat-ion exchange membranes defining a chamber for the AX-REED liquid; and
2. b. two further chambers for a second dialysis liquid, wherein said two further chambers are positioned adjacent to the chamber for the AX-REED liquid on opposing sides and wherein said two further chamber may be connected

ii. a set of end membranes

iii. means for applying an electric field over the membrane stack by means of at least two electrodes

iv. means for reversing the direction of the electric field within said membrane stack

and wherein the removal involves the steps of

1. i) inserting the AX-REED liquid (i.e. the liquid obtained after AX-REED treatment) into the chamber for the AX-REED liquid; and
2. ii) inserting a second dialysis liquid in the two further chambers for the second dialysis liquid; and
3. iii) applying an electric field over the membrane stack;
4. iv) incubating said AX-REED liquid in said chamber, whereby the direction of electric field is reversed at intervals

[0365] In embodiments of the disclosure, where the AX-REED and the CX-REED is performed simultaneously in parallel, then at the onset of the procedure the liquid to be treated by CX-REED is in fact the starting liquid. Thus, the CX-REED membrane stack may then contain

v) at least one cell consisting of:

1. a. two cat-ion exchange membranes defining a chamber for the starting liquid; and
2. b. two further chambers for a second dialysis liquid, wherein said two further chambers are positioned adjacent to the chamber for the starting liquid on opposing sides and wherein said two further chamber may be connected

v. a set of end membranes

vi. means for applying an electric field over the membrane stack by means of at least two electrodes

vii. means for reversing the direction of the electric field within said membrane stack

and wherein the removal involves the steps of

1. i) inserting the starting liquid into the chamber for the AX-REED liquid; and
2. ii) inserting a second dialysis liquid in the two further chambers for the second dialysis liquid; and
3. iii) applying an electric field over the membrane stack;
4. iv) incubating said starting liquid in said chamber, whereby the direction of electric field is reversed at intervals

[0366] In some embodiments of the disclosure AX-REED is performed first and then AX-REED and CX-REED are performed simultaneously in parallel. In these embodiments, then at the onset of the the CX-REED treatment, then partly AX-REED treated liquid is inserted into the chamber for starting liquid or the chamber for AX-REED liquid. For the sake of simplicity, then the chamber may never-the-less be referred to as chamber for starting liquid or chamber for AX-REED liquid.

[0367] As described above the methods of the disclosure contains a step of removing one or more acid acidic ions from the liquid through an AX-REED membrane stack into a dialysis liquid, which typically contains a base. This may result in the basic cation being transferred from the dialysis liquid into the starting liquid and therefore the method may contain the step e), wherein at least some of said cation is removed from the liquid.

[0368] This step may also be referred to as CX-REED treatment. As described above step e) may be performed using a REED equipment. The liquid resulting from step d) of the method of the disclosure is herein referred to as "AX-REED liquid". The AX-REED liquid may be used as starting liquid for step e). Alternatively, a partly AX-REED treated liquid may be used as starting liquid for step e). It is also possible that the starting liquid for step d) is also the starting liquid for step e), when steps d) and e) are performed simultaneously.

[0369] Step e) of the methods of the disclosure involves use of one or more CX-REED membrane stacks, wherein each of said membrane stacks contains

v) at least one cell consisting of:

1. 1. two an-ion exchange membranes defining a chamber for the AX-REED liquid, the partly AX-REED treated liquid or the starting liquid; and
2. 2. two further chambers for a second dialysis liquid, wherein said two further chambers are positioned adjacent to the chamber for the starting liquid on opposing sides and wherein said two further chambers may be connected

viii) a set of end membranes

ix) means for applying an electric field over the membrane stack by means of at least two electrodes

x) means for reversing the direction of the electric field within said membrane stack.

[0370] Thus regardless of whether the electric field ions will be able to move from the chamber defining the AX-REED liquid, the partly AX-REED treated liquid or the starting liquid into either of the chambers for the second dialysis liquid.

[0371] Each CX-REED membrane stack may comprise more than one cell as identified above. For example each CX-REED membrane stack may comprise in the range of 2 to 100 cells, such as in the range of 2 to 50 cells, such as in the range of 2 to 25 cells.

[0372] The removal of the cation typically involves the steps of

1. i) inserting the AX-REED liquid, the partly AX-REED treated liquid or the starting liquid into the chamber for the starting liquid, wherein said AX-REED liquid is obtained by AX-REED treatment of the starting liquid as described above in the section "AX-REED"; and
2. ii) inserting a second dialysis liquid in the two further chambers for the second dialysis liquid, wherein the second dialysis may be any of the second dialysis liquids described herein below; and
3. iii) applying an electric field over the membrane stack;
4. iv) incubating said starting liquid in said chamber, whereby the direction of electric field is reversed at intervals.

[0373] CX-REED may be performed under circulation meaning that after incubation of the AX-REED liquid, the partly AX-REED treated liquid or the starting liquid in said chamber the resulting liquid may be removed from the chamber and later inserted into another chamber for the AX-REED liquid, the partly AX-REED treated liquid or the starting liquid or even into the same chamber for the AX-REED liquid or starting liquid. When inserted into the same chamber, then frequently the second dialysis liquid in said two further chambers has been exchanged for a fresh second dialysis liquid.

[0374] The cation to be removed may for example be any cation, but typically it will be one or more cations introduced into the AX-REED liquid from the dialysis liquid during AX-REED treatment. Thus, the cation may for example be any of the cations of a base, which may be included in the dialysis liquid as described herein above in the section "AX-REED".

[0375] During removal of said cation, the two membranes surrounding the chamber for the AX-REED liquid or starting liquid either facilitates the transport of ions out of the AX-REED liquid or into the AX-REED liquid from the second dialysis liquid.

[0376] The direction of the electric field is changed at intervals in a manner similar to what is described herein above for AX-REED.

[0377] The methods of the disclosure may involve use of more than one CX-REED membrane stack. The membrane stacks may be stacked (commonly separated by membrane spacers) on top of each other or side by side until sufficient membrane separation area is achieved to obtain a desired retention time. For feasible handling, operational, and maintenance purposes, the membrane stacks may be operated in several separate, practically sized membrane stacks, each with its own set of flow connections and electrodes, but with the same separation function. These stacks are operated together in parallel or serial or some combination thereof as part of the same separation system. It is advantageous to operate with multiple CX-REED membrane stacks, when more than one set of electrodes are used. The number of CX-REED membrane stacks may thus vary from 2 to several hundred depending on the process in question, but are typically in the range 2-50 CX-REED membrane stacks, more typically in the range of 4-20 membrane stacks.

[0378] The second dialysis liquid to be used with CX-REED according to the disclosure may be any acidic solution. Typically it is an aqueous solution of a H-anion, wherein the anion typically is an inorganic anion. Thus, for example the second dialysis liquid may comprise one or more acids selected from the group consisting of H_3PO_4 , HNO_3 and H_2SO_4 . Preferably, the second dialysis liquid comprises H_3PO_4 . The second dialysis liquid will typically contain said acid in a concentration in the range of 5 to 90%, preferably in the range of 10 to 90%, more preferably in the range of 20 to 80%, yet more preferably in the range of 30 to 80%, for example in the range of 40 to 80%, such as in the range of 50 to 80%, for example in the range of 60 to 80%. The percentages are provided as w/w.

[0379] In the case of CX-REED, cations are extracted through one cation-exchange membrane of each cell of the CX-REED membrane stack(s), while typically H^+ ions enter through the opposite cation-exchange membrane. When the direction of the electrical field is reversed, the extracted cations inside the first mentioned membrane is pushed back into the AX-REED liquid, before H^+ ions starts entering the AX-REED liquid. In order to spread out the instability effects and reduce the overall impact of current reversals with more than one membrane stack, the reversals of the electric field on each separate stack are preferably performed asynchronously. Thus, it is preferred with the disclosure that more than one CX-REED membrane stack is employed and that the electric field on each separate stack is reversed asynchronously. Even though the intervals for reversing the electrical field of each stack are typically of similar length, the timing of the reversals are dispersed for best process stability effect.

[0380] In an embodiment of the disclosure the direction of the electric field within any first membrane stack is reversed at substantially regular dispersal intervals relative to the reversals for any second or further membrane stack.

[0381] The interval length between current reversals for a stack is typically chosen with regard to the build-up of membrane fouling. Typically, said intervals within any one CX-REED stack may be in the range 5-6000 seconds, preferably 8-3000 seconds, more preferably 10-2000 seconds and even more preferably 100-1500 seconds.

In another embodiment of the disclosure the direction of the electric field within any first membrane stack is reversed at dispersal intervals of substantially even length relative to the reversals for any second or further membrane stack in order to maximise the time between a current reversal of any first CX-REED stack and any second or further CX-REED stack in the same process. With the same dispersal interval length between current reversals, i.e. where these reversals are dispersed evenly the connected bioreactor will experience a reduced impact, but much more often.

[0382] In an embodiment of the disclosure the intensity of the applied electric field is adjusted in response to the pH, target ion concentration or conductivity of said liquid composition. By increasing the intensity of the electric field, the ion exchange increases in the CX-REED system, and vice versa. Online, semi-online (e.g. time-delayed) or secondary (e.g. using online conductivity or turbidity measurements for estimating target ion concentration) measurements of the process parameters being regulated are input in a control regulation mechanism e.g. PID-control software, which in turn regulates the output of the power supplies to the CX-REED electrodes.

[0383] The reversal of electrical field is not the only effect, which can introduce deviations in process control. For optimal control of process parameters, it may be advantageous to control the concentration of various ions in the second dialysis liquid as well as the flow and temperature and mode of operation.

[0384] If multiple stacks are used it is possible to set up the flow of the second dialysis liquid either in parallel, or in serial mode with or without booster pumps between stacks, in a similar fashion as with the AX-REED liquid.

[0385] In general the Cation-Exchange REED (CX-REED) serves to replace cations with hydrogen ions.

[0386] The cation exchange membranes may be any useful cation exchange membranes. The size of the membranes may be selected in order to achieve a suitable retention time. For calculating the retention time, the total area of the anion membranes used is of interest. Accordingly, if the method employs use of many membrane stacks and/or if each membrane stack contains many cells, then the area of each membrane may be reduced.

[0387] Non-limiting examples of useful CX-membranes includes Nafion N117 (cation)(Dupont) and Fumatech FAB (cation)(Fumatech, Germany).

[0388] Non-limiting examples of useful methods and equipment for performing AX-REED are also described in European patent applications EP 1 347 823, EP2349541 and EP2349540.

[0389] In general, the CX-REED is performed in order to remove at least part of one cation from a liquid, and in particular from the AX-REED liquid. The CX-REED is performed for at time sufficient to remove a desired amount of said at least one cation. In one preferred embodiment of the disclosure step e) is performed in a manner so that the CX-REED liquid produced has a conductivity of at the most 7 mS/cm, preferably of at the most 6 mS/cm, even more preferably of at the most 5 mS/cm, for example in the range of 3 to 5 mS/cm. If the liquid has a higher conductivity, then CX-REED may be continued until the CX-REED liquid has the desired conductivity. In general, a higher conductivity than 5 mS/cm is less desirable in the CX-REED liquid, because this may cause a salty taste. When steps d) and e) are performed simultaneously it is preferred that step e) is performed in a manner so that the REED liquid produced has a conductivity of at the most 7 mS/cm, preferably of at the most 6 mS/cm, even more preferably of at the most 5 mS/cm, for example in the range of 3 to 5 mS/cm. If the liquid has a higher conductivity, then CX-REED may be continued until the REED liquid has the desired conductivity. In general, a higher conductivity than 5 mS/cm is less desirable in the REED liquid, because this may cause a salty taste.

[0390] In embodiments of the disclosure wherein the starting liquid comprises a cereal extract, then it is preferred that step e) is performed in a manner so that the CX-REED liquid or the REED liquid produced has a conductivity of at the most 7 mS/cm, preferably of at the most 6 mS/cm, even more preferably of at the most 5 mS/cm, yet more preferably in the range of 3 to 5 mS/cm, such as in the range of 4 to 5 mS/cm, for example approx. 4.5.

[0391] In embodiments of the disclosure wherein the starting liquid comprises a fruit juice or a fruit extract, then in general it is preferred that the conductivity is low. Thus, in these embodiments it is preferred that step e) is performed in a manner so that the CX-REED liquid or the REED liquid produced has a conductivity of at the most 6 mS/cm, preferably of at the most 5 mS/cm, even more preferably of at the most 4 mS/cm, yet more preferably in the range of 2 to 4 mS/cm, such as in the range of 3 to 4 mS/cm, for example approx. 3.5.

Contact time

[0392] The contact time is a figure, which is useful for managing the REED process, According to the disclosure the contact time in relation to AX-REED is calculated as

$$\text{(Total area of an-ion exchange membranes (cm}^2\text{)/volume of starting liquid (cm}^3\text{))} \times \text{time (h)}.$$

[0393] The total area of an-ion exchange membranes is the total area of all an-ion exchange membranes of all cells in all membrane stacks used for the AX-REED. The contact time should be selected in order to arrive at a beverage with a palatable ration of sugar to organic acid. Preferably, the ratio of sugar to organic acid of the beverages of the disclosure is the ration described in the section "Ratio of sugar to organic acid" herein.

[0394] In embodiments of the disclosure, where the methods comprises step c), then it is generally preferred that the contact time is relatively long, whereas the contact time may be much shorter in embodiments of the disclosure lacking step c).

[0395] Furthermore, a long contact time may unfortunately lead to accumulation of off-flavours and accordingly the contact time should preferably also not be too long.

[0396] In one embodiment of the disclosure the contact time in relation to AX-REED is in the range of 0.5 to 100 h, such as 1 to 50 h, for example 1 to 10 h. In embodiments of the disclosure relating to any of the methods described in the sections "Method of producing a fermented beverage" and "Methods of producing a beverage with enzymatic conversion of sugar" then the contact time in general may be in the range of 5 to 10 h.

[0397] According to the disclosure the contact time in relation to CX-REED is calculated as $(\text{Total area of cat-ion exchange membranes (cm}^2\text{)}/\text{volume of AX-REED liquid (cm}^3\text{)}) \times \text{time (h)}$.

[0398] The total area of cat-ion exchange membranes is the total area of all cat-ion exchange membranes of all cells in all membrane stacks used for the CX-REED. The contact time of CX-REED is in general much lower than the contact time for AX-REED.

[0399] In one embodiment of the disclosure the contact time in relation to CX-REED is in the range of 0.01 to 10, such as 0.05 to 5, for example 0.1 to 1.

Additional compound

[0400] The methods of the disclosure may comprise a step f) of adding one or more additional compound(s). The additional compound may for example be a flavor compound, a preservative or a functional ingredient.

[0401] The flavor compound may be any of the flavor compounds described herein below in the section "Flavor compound".

[0402] Functional ingredients may be any ingredient added to obtain a given function. Preferably a functional ingredient renders the beverage more healthy. Non-limiting examples of functional ingredients includes soluble fibres, proteins, added vitamins or minerals.

[0403] The preservative may be any food grade preservative, for example it may be benzoic acid, sorbic acid, or salts thereof.

[0404] The additional compound may also be CO₂. In particular, CO₂ may be added to obtain

a carbonated beverage.

Flavour compound

[0405] The flavour compound to be used with the present disclosure may be any useful flavour compound. The flavour compound may for example be selected from the group consisting of aromas, plant extracts, plant concentrates, plant parts and herbal infusions.

[0406] Thus, the flavour compound may for example be an aroma. Aromas are typically organic compounds, for example they may be plant secondary metabolites. The aroma may be any aroma, for example a fruit aroma or vanilla aroma.

[0407] The plant extract may for example be a herbal extract. Non-limiting examples of herbal extracts includes an extract of green tea, black tea, rooibos, peppermint or hops. The plant extract may also be a flower extract. Non limiting examples of flower extracts includes hibiscus camomile, elderflower, lavender or linden flower.

[0408] The plant extract may also be a fruit extract. Plant parts may for example be dried or fresh herbs, such as hops pellets, dried of fresh flowers or fruits.

[0409] The plant concentrate may be a fruit concentrate, for example a fruit juice, which has been concentrated by removal of water.

[0410] Non-limiting examples of fruits useful for fruit aroma, fruit extract or fruit concentrates include orange, apple, banana, lemon, passion fruit, mango, pineapple, pears, kumquats or pomelo,

[0411] The flavor compound may also be tonic.

Sequence listing

[0412]

SEQ ID NO:1	Amino acid sequence of glucan 1,4-alpha-glucosidase of Aspergillus niger
SEQ ID NO:2	Amino acid sequence of glucan 1,4-alpha-glucosidase of Aspergillus oryzae (strain ATCC 42149 / RIB 40)
SEQ ID NO:3	Amino acid sequence of glucan 1,4-alpha-glucosidase of Rhizopus oryzae
SEQ ID NO:4	Amino acid sequence of alpha-amylase of Aspergillus niger
SEQ ID	Amino acid sequence of alpha-amylase of Aspergillus oryzae

NO:5	
SEQ ID NO:6	Amino acid sequence of alpha-amylase of <i>Rhizopus oryzae</i>
SEQ ID NO:7	Amino acid sequence of pullulanase of <i>Bacillus subtilis</i>
SEQ ID NO:8	Amino acid sequence of pullulanase of <i>Bacillus cereus</i> (strain ZK / E33L)
SEQ ID NO:9	Amino acid sequence of pullulanase of <i>Lactobacillus acidophilus</i> (strain ATCC 700396 / NCK56 / N2 / NCFM)
SEQ ID NO:10	Amino acid sequence of glucose oxidase of <i>Aspergillus niger</i>
SEQ ID NO:11	Amino acid sequence of glucose oxidase of <i>Penicillium amagasakiense</i>
SEQ ID NO:12	Amino acid sequence of catalase of <i>Aspergillus niger</i>

Examples

[0413] The disclosure is further illustrated by the following examples. Examples not falling under the scope of the appended claims do not form part of the invention.

Example 1

[0414] Standard wort of 14,85%P was produced as described herein below in Example 3. 7,5 L standard wort was fermented, at 30°C, by a mixture of *Lactobacillus sanfranciscensis* and *Lactococcus lactis* in a REED rig (Jurag Separation, Denmark). The REED rig was equipped with both an AX-REED and a CX-REED membrane stack. After fermentation to an end-point of pH 3.9, the ferment was allowed to stand at 4°C for 36 hours, after which the bulk of the liquid was decanted from the sedimented bacteria. Similar tests with each of the bacteria alone were conducted. The supernatants were filtered through a Seitz EK filter, carbonated, pasteurised, and their flavour evaluated. Lactic acid bacteria were harvested by centrifuging the sediment at 8000 rpm for 10 minutes, and then left for a further 36 hours before repeating the REED controlled fermentation. The consumption of maltose and glucose by the bacteria in the first and second fermentations is depicted in figure 2.

[0415] Although the maltose content of the wort differed in the two fermentations, so the start and end points were off-set, the performance of the bacteria was very similar in both cases, despite the inoculum for the second one having been cropped and stored at low pH for 72 hours. This ability to metabolise maltose shows that the *Lactobacillus sanfranciscensis* is considerably resistant to the treatment it received. The decline in the speed with which glucose

was removed from the second fermentation suggests that *Lactococcus lactis* is more sensitive to these conditions.

[0416] The genome sequence of *Lactobacillus sanfranciscensis* has been published, and reveals that this organism is completely devoid of extracellular proteases. Interestingly, the flavour obtained from wort fermented by *Lactobacillus sanfranciscensis* lacks the unwanted peptone notes, characteristic of other lactic acid fermentations.

Example 2

[0417] This example describes production of a fermentation process for wort, which does not produce alcohol, but adjusts the sweetness to acidity imbalance in this raw material, to produce a refreshing drinks base, which is rich in natural vitamins and minerals, and low in calories.

[0418] The resulting drinks base can be used as a beverage per se or various flavor compounds can be added before consumption.

[0419] *Lactobacillus sanfranciscensis* was cultivated in medium containing maltose as the only carbon source. The maltose was labeled with ^{13}C NMR. It was shown that *Lactobacillus sanfranciscensis* was unable to form glycolytic metabolites from glucose when immediately transferred to this carbon source. *Lactococcus lactis*, which cannot grow on maltose, when fed ^{13}C -labelled glucose, rapidly produces NMR signals which are attributable to glycolytic metabolites.

[0420] The provision of maltose, ^{13}C -labelled on the reducing end, to a mixture of *Lactobacillus sanfranciscensis* and *Lactococcus lactis* would therefore only produce an NMR spectrum attributable to glycolytic metabolites, if the glucose released from maltose by phosphorolysis in *Lactobacillus sanfranciscensis* is metabolised by *Lactococcus lactis*. This was confirmed by the experiment, which yielded an NMR spectrum attributable to glycolytic metabolites.

Example 3

Production of wort

[0421] A standard wort of 14.5%P was produced from 39,8 kg of a standard Pilsner malt, which was mashed-in with 108.4 L standard brewing water at 65°C. Just after mixing milled malt with water, a commercial enzyme preparation containing an arabinoxylanase activity was added in order to facilitate filtration of the finished wort. Calcium chloride was also added at this

stage, and pH was adjusted to ca. 5,4 by addition of phosphoric acid. After 60 minutes at 65°C, the temperature was gradually increased to 78°C during a 15-minutes period, and finally kept at 78°C for 5 minutes. The mash was then filtrated and sparged, resulting in a total volume of 212 L prior to boiling. The wort was adjusted to pH ca. 5.2 by addition of phosphoric acid, and calcium chloride was added. The wort was then boiled 70 minutes. During this period, ca. 5% water evaporated, leaving 200 L boiled wort. After a whirlpool process to remove sediments, the boiled wort was filled into kegs and kept at 5°C until REED processing. This wort, and worts produced in essentially the same way, is herein also referred to as "standard wort". The contents of fermentable sugars and organic acids in the standard wort are shown in table 2.

[0422] Detection and quantification of 5 fermentable carbohydrates (glucose, fructose, sucrose, maltose, maltotriose) was performed by high performance anion-exchange chromatography (HPAEC) using DIONEX ICS-3000 (ThermoScientific) connected to a PC equipped with the software Chromeleon. The hydroxyl-groups of carbohydrates are ionized to oxonium ions at pH 12-14 which allow separation of carbohydrates by anion exchange chromatography. Detection is then performed by Pulsed Electrochemical Detection (PED) (Pulsed Amperometric Detection (PAD)) using integrated amperometry. Quantification was allowed by use of HPLC-grade calibration standards: glucose (Sigma G-8270), fructose (Sigma F-0127), sucrose (Sigma S-9378), maltose (Sigma M-5885), maltotriose (Sigma M-8378).

[0423] Organic acids were determined using HPLC equipped with a "Prevail organic acid column" 150 x 4,6mm. UV Detection was performed at 210nm. As mobile phase 25mM KH_2PO_4 pH 2,5 was used. As organic phase acetonitrile was used. The separation was done linearly with 100% Mobile phase (total separation time 7.5 min.).

REED fermentation

[0424] 250 g 80% lactic acid and 237 g 46% potassium hydroxide was added to 37.5 L standard wort to increase pH and conductivity in the starting liquid. This starting liquid was inoculated with commercial, frozen pure cultures of *Lactococcus lactis* and *Lactobacillus sanfranciscensis* (200 g and 400 g, respectively), and left to ferment, at 30°C. Acids produced during the fermentation were removed by the REED equipment, set up with Ionics AR103/Nafion N117 AX-membranes (12 cells pairs, 2 mm thickness, 915 cm² active areas per cell pair) and Nafion N117/Fumatech FAB CX-membranes (10 cell pairs, 2 mm thickness, 915 cm² active area per cell pair). AX-REED dialysis liquid was KOH (46%). CX-REED second dialysis liquid was H_3PO_4 (75%). Both dialysis liquids were only passed through the system once (single passage). The AX-REED and CX-REED membrane stacks were connected in parallel, however the CX-REED membrane stack was only operated in the last part of the process. An overview of the REED equipment is shown in figure 1. The fermentation was terminated after 25 hours, when the liquid had a pleasant balance between sweetness and acidity. The analytical results are shown in table 2.

Table 2

	Standard wort	REED product
<i>Sugars, g/L:</i>		
Glucose	9,4	Trace
Fructose	Trace	0
Sucrose	5,0	0
Maltose	65,3	21,7
Maltotriose	15,1	7,2
<i>Organic acids, mg/L:</i>		
Tartaric	74	0
Malic	43	7
Lactic	0	484
Acetic	126	15
Citric	539	0
Succinic	475	113
Propionic	0	0
<i>pH:</i>	5,20	4,37

Example 4

[0425] A wort of 14,5 %P, with a high content of glucose, was produced by mashing a standard Pilsner malt together with commercial brewing enzymes. 43,8 kg Pilsner malt was mashed-in with 131 L standard brewing water at 63°C. Just after mixing milled malt with water, commercial enzyme preparations containing alpha-glucosidase, α -amylase, and limit-dextrinase activities were added, which are capable of converting carbohydrates and oligosaccharides to glucose. Calcium chloride was also added, and pH was adjusted to approx. 5.2 by addition of phosphoric acid. After 30 minutes at 63°C, the temperature was increased to 70°C at a rate of 1°C/minute, kept at 70°C for 60 minutes, increased to 78°C at a rate of 1°C/minute, and finally kept at 78°C for 5 minutes. The mash was then filtrated and sparged, resulting in a total volume of 233 L prior to boiling. The sweet wort was adjusted to pH ca. 5,2 by addition of phosphoric acid, and calcium chloride was added. The wort was then boiled 70 minutes. During this period, ca. 5% water evaporated, leaving 220 L boiled wort. After a whirlpool process to remove sediments, the boiled wort was filled into kegs and kept at 5°C until REED processing. This wort, and worts produced in essentially the same way, may herein also be referred to as "glucose wort". The contents of sugars and organic acids in the glucose

wort were determined as described in Example 3 and the results are shown in table 3.

REED fermentation

[0426] 39,4 L glucose wort prepared as described above and 250 g 80% lactic acid and 227 g 46% potassium hydroxide was added to increase pH and conductivity in the starting liquid. This starting liquid was inoculated with 260 g of a commercial, frozen pure culture of *Lactococcus lactis* and left to ferment, at 25°C. Acids produced during the fermentation were removed by the REED equipment, set up with an AX-REED membrane stack and a CX-REED membrane stack as follows:

AX-REED membrane stack:

12 cell pairs

Membranes: Ionics AR103, Nafion N117

Total membrane area: 10980 cm²

CX-REED membrane stack:

10 cell pairs

Membranes: Nafion N117, Fumatech FAB

Total membrane area 9150 cm²

[0427] The AX-REED dialysis liquid was KOH (46%). The CX-REED second dialysis liquid was H₃PO₄ (75%). Both dialysis liquids were only passed through the system once (single passage). The AX-REED and CX-REED membrane stacks were connected in parallel, however the CX-REED membrane stack was only operated in the last part of the process. The fermentation was terminated after 23 hours, when the resulting liquid (REED liquid) had a pleasant balance between sweetness and acidity. The analytical results for the REED product are shown in table 3.

[0428] After REED fermentation, part of the REED liquid was flavoured by addition of pellets of an aroma hop variety. The pellets were left in the liquid at 14°C for 20 hours and then removed. The flavoured liquid had a pleasant and characteristic hop aroma.

Table 3

	Glucose wort	REED liquid
Sugars, g/L:		

	Glucose wort	REED liquid
Glucose	141,4	22,2
Fructose	Trace	Trace
Sucrose	5,7	3,5
Maltose	Trace	Trace
Maltotriose	0	0
<i>Organic acids, mg/L:</i>		
Tartaric	29	0
Malic	6	0
Lactic	0	773
Acetic	116	0
Citric	485	0
Succinic	348	11
Propionic	0	0
<i>pH:</i>	5,20	4,35

Example 5

[0429] Compared to orange juice, which has a pleasant balance between sweetness and acidity, lemon juice has a much higher content of organic acids, and a lower content of sugars. The dominating organic acid in lemon juice is citric acid, but relatively high levels of malic acid and ascorbic acid (vitamin C) are also found. Addition of high amounts of extra sugar and dilution with water is therefore the traditional way to produce palatable beverages based on lemon juice. However, the present disclosure provides a method of reduction of acidity, which result in production of pleasant lemon based juices with same low acidity as orange juices, but lower calorie content due to lower content of natural sugars.

[0430] Lemon juice was obtained by squeezing fresh lemons. In two separate experiments, 25 L juice was recirculated, at 25°C, through the REED equipment, set up with Fumatech FAB/Nafion N117 AX-membranes and Nafion N117/Fumatech FAB CX-membranes. The AX-REED and the CX-REED membrane stack were connected in parallel and operated in a manner, so that pH never exceeded approx. 3.5. An overview of the REED equipment is shown in figure 1. In both trials, a gradual decrease of organic acids was observed. In Trial 8, the treatment was allowed to proceed for 4.5 hours. This reduced the contents of organic acids to very low levels (fig. 3A) and demonstrated the efficiency of the process, but the lack of acidity made the resulting liquid rather bland. In Trial 9, the treatment was terminated after 3.5 hours

(fig. 3B). The resulting liquid from this trial had an acidity comparable to fresh orange juice and a mild sweetness. The REED treatment reduced the contents of both citric, malic, and ascorbic acid. However, while only about a third of the citric acid and malic acid remained after treatment for 3.5 hours, more than half of the original content of ascorbic acid, important for its vitamin function, was left in the REED liquid from Trial 9 (table 4).

Table 4

	Citric acid	Malic acid	Ascorbic acid	pH
	g/L	g/L	g/L	
Orange, fresh	24	0,93	0,42	3,54
Lemon, fresh	61	2,08	0,49	2,37
Lemon, after REED	22	0,57	0,27	3,52

Example 6

[0431] Rosehips are well-known in the Scandinavian countries and Russia for their content of ascorbic acid (vitamin C) and other antioxidants. However, rosehips also contain citric acid, and extracts are therefore rather acidic and unpalatable. The present disclosure provides methods for reducing the acidity of rosehip extracts, to produce palatable liquids which retain a high content of vitamins and antioxidants.

[0432] An extract of rosehips was prepared by heating 20 L water to 50°C and adding 4 kg of dried, milled rosehips. After dispersing the rosehip chips by shaking, the mixture was left at 50°C, with occasional stirring. One hour after mixing, a commercial pectinase preparation, high in arabanase activity, was added, and the mixture was left for further 16 hours at 50°C. The rosehip chips were then removed by filtration and sparged with additional water. After combining the first filtrate with sparged liquid, a total of 22 L liquid was obtained. The extract was dark reddish-brown, acidic, and very astringent.

[0433] The 22-L batch of rosehip extract was recirculated, at 25°C, through the REED equipment, set up with Fumatech FAB/Nafion N117 AX-membranes and Nafion N117/Fumatech FAB CX-membranes. The AX-REED and the CX-REED membrane stacks were connected in parallel. An overview of the REED equipment is shown in figure 1. After approx. 2 hours, the treatment was terminated. When tasted, the acidity of the resulting liquid was found to be significantly reduced, but some astringency remained. Chemical analysis, by HPLC, showed that the content of citric acid had been reduced by 50% by the short REED treatment, from 1.34 g/L in the crude rosehip extract to 0.65 g/L after REED treatment. Other organic acids were generally reduced to the same extent (table 5).

[0434] In order to reduce astringency, a commercial preparation of PVPP (polyvinyl-polypyrrolidone, routinely used in beer manufacturing to reduce the content of polyphenols in

beer) was added and left in contact with the REED product at 5°C for 16 hours. PVPP was then removed by filtration. The resulting liquid was still dark reddish-brown, but with moderate acidity and astringency. The antioxidant activity, measured by the ORAC-method essentially as described in Dávalos et al., 2004, (vide supra), was still very high (table 5).

Table 5

	Crude rosehip extract	After REED treatment	After REED + PVPP
<i>Organic acids (mg/L):</i>			
Tartaric	0	0	n.d.
Malic	211	101	n.d.
Ascorbic	32	30	n.d.
Lactic	129	89	n.d.
Acetic	95	0	n.d.
Citric	1335	652	n.d.
Succinic	59	37	n.d.
Propionic	0	0	n.d.
<i>Antioxidant activity (trolox-eq./mL)</i>	n.d.	n.d.	120

Example 7

[0435] An extract of blackcurrants was prepared by blending frozen berries with an equal weight of water, followed by removal of pulp by centrifugation. The resulting liquid was rather acidic in taste.

[0436] An extract of sea buckthorne was also prepared by blending frozen berries with an equal weight of water, followed by removal of pulp by centrifugation.

[0437] The berry extracts were each treated in a REED equipment for 2.5-3 hours using a "feed-and-bleed" set-up, where only a part of the total volume of juice (generally 7-8 L) was initially fed into the REED equipment and deacidified. Then a small volume was then taken out via a pump and replaced with fresh untreated juice, and this was continued until all the juice had been treated. The REED equipment was set up with Ionics/Nafion AX membranes. The pH was initially raised from the starting pH and up to pH 4 or slightly less, and then pH was maintained almost constant during the remainder of the process by continuously adding fresh berry extract of low pH and removal of acidic ions by AX-REED.

[0438] The mineral content in the juices before and after REED treatment is shown in Tables 6 and 7. Before analysis all samples were filtered.

Table 6 Starting liquid: frozen sea buckthorne berries blended with water, centrifuged.

Mineral	SBB: conc. before REED	SBB: conc. after REED	SBB: Recovery
	mg/L	mg/L	%
Calcium	63	65	104
Magnesium	43	43	101
Iron	1,0	0,90	88
Silica	5,6	5,4	97

Table 7 Starting liquid: frozen blackcurrants blended with water, centrifuged.

Mineral	BC-2: conc. before REED	BC-2: conc. after REED	BC-2: recovery
	mg/L	mg/L	%
Calcium	167	152	91
Magnesium	91	78	86
Iron	1,1	0,92	83
Silica	6,8	7,8	115

[0439] Sugar content was determined as described in Example 3, and antioxidant levels were determined essentially as described in Dávalos et al., 2004, (vide supra). The results are shown in table 8.

[0440] The REED-treated blackcurrant juice was evaluated by a trained taste panel together with two commercially available blackcurrant beverages, Ribena "classic" (sweetened with sugar) and Ribena "light" (sweetened with artificial sweeteners). The taste panel accorded a total flavor score to the three beverages using a scale from 0 to 9, where 0 designates an undrinkable product and 9 an extremely pleasant product. Furthermore sugar content, and antioxidant content was determined. The results are shown in Table 8.

[0441] The REED-treated black currant juice was less sweet and somewhat more acidic than the two commercial products. However, the REED product scored higher in fruity/estery aroma and in fruit/berry taste, and scored less in artificial taste. Total flavour score for the REED product was almost the same as for Ribena "classic" and Ribena "light".

Table 8. Comparison of REED-treated blackcurrant juice with commercial blackcurrant beverages.

	Blackcurrant juice after REED	Ribena "classic"	Ribena "light"
Blackcurrant juice in RTD	50%	5%	7%
Sugars, g/100 mL	4,8	10,5	0,5
Other Sweeteners	none	None	aspartame,

	Blackcurrent juice after REED	Ribena "classic"	Ribena "light"
			acesulfame K
kcal/100 mL	20	44	3
Added vitamin C	no	Yes	Yes
Antioxidants, oracs/100 mL	2000	400	350
Total flavor score	5.9	6.0	6.2

Example 7

[0442] Glucose worts were prepared essentially as described in Example 4 and subjected to REED fermentation also essentially as described in Example 4 with the following amendments. No lactic acid was added to the glucose wort and only 200 g KOH. In trial 40 the fermentation was terminated after 22.5 hours. In trial 54 the the fermentation was terminated after 24.5 hours, when the glucose level was approx. 40 g/L. In trial 55 the fermentation was terminated after 50 hours, when the glucose level was 5 g/L. In the individual trials the mineral content of the glucose wort and of the products of the REED fermentations (REED liquids) were determined and the percent recovery calculated. The results are shown in Table 9. As can be seen, the level of both calcium, magnesium and iron is maintained after REED fermentation for up to 50 hours.

Table 9 Percent mineral recovery after REED fermentation

trial no.	recovery, %		
	Ca	Mg	Fe
40	96	69	95
54	92	68	91
55	116	82	109

Example 8

[0443] In order to establish a pleasant balance between sweetness and acidity in a beverage, a REED-assisted fermentation was made, where glucose wort, prepared essentially as described in Example 4 was fermented with *Lactococcus lactis* in the REED equipment essentially as described in Example 4 except for the following amendments. No lactic acid was added to the glucose wort and only 200 g KOH. The fermentation was carried out until the glucose content in the resulting liquid (the REED liquid) was very low, ca. 2 g/L, and the lactic acid content was ca. 5 g/L. The liquid also contained minor amounts of fructose (< 1 g/L),

sucrose (ca. 3 g/L), maltose (ca. 2 g/L), and maltotriose (< 1 g/L). Thus, the total sugar content of the resulting base was approx. 7 g/L. This base had a neutral flavor.

[0444] Three beverages, differing in sweetness but otherwise practically identical, were prepared from the base by adding glucose to obtain final concentrations of 22, 37, and 52 g/L. In order to flavor the beverages, hop-pellets were added at a dose of 2 g/L and left in the liquids for 24 hours at 5-8°C. The hop pellets were then removed, and the beverages were carbonized.

[0445] The three beverages were then tasted by a total of 75 persons. 34 persons were women, and 41 were men; 33 were aged 20-40, and 42 were aged 41-65. At the tasting session, all three samples were served at the same time to each person. The participants were asked to evaluate the three beverages as "non-alcoholic hop-flavoured softdrinks for adults", informed about the glucose content of the beverages, and then asked to select their favorite. This survey showed a very clear preference for the beverage with a glucose content of 37 g/L. 41 persons preferred 37 g/L, while 18 preferred 22 g/L and 16 preferred 57 g/L. This preference was not significantly influenced by age or gender. The results are shown in figure 5.

[0446] The three beverages were also evaluated by a taste panel trained in beer evaluation. The panelists were asked to score "beer flavour attributes" on a scale from 0-5, where 0 indicates absence of a flavour and 5 a very intense flavour. The panellists were also asked to assign a total flavour score for each beverage on a scale from 0-9, where 0 is undrinkable and 9 is excellent. The panel gave almost equal scores for glucose 22 g/L and 37 g/L, but glucose 52 g/L was judged as markedly oversweet. The beverages with 22 g/L and 37 g/L sugar were also considered significantly better in the total flavour test. Thus total flavour scores for 22, 37, and 52 g/L glucose were 6,0, 6,1 and 5,5, respectively.

Example 9

[0447] This examples demonstrates the particularly pleasant taste of a beverage prepared by REED-assisted bacterial fermentation of glucose wort. Glucose wort, prepared essentially as described in example 4, was fermented with *Lactococcus lactis* in REED equipment essentially as described in Example 4, except that the fermentation was interrupted when the glucose content in the liquid was ca. 37 g/L, the lactic acid content was 6,7 g/L, and pH was 4,35. Also no lactic acid was added to the glucose wort and only 200 g KOH. The liquid also contained minor amounts of fructose (< 1 g/L), sucrose (ca. 3 g/L), maltose (ca. 3 g/L), and maltotriose (< 1 g/L). This base had a mild sweetness and a mild acidity. In order to flavour the liquid, hop pellets were added at a dose of 2 g/L and left in the liquid for 24 hours at 5-8°C. The hop pellets were then removed, and the liquid was carbonized. This beverage was called Beverage A.

[0448] Another beverage was prepared from glucose wort, prepared essentially as described in example 4 To obtain pH, sweetness, and acidity comparable to the REED-based beverage,

the glucose wort was diluted with water, and a mixture of lactic acid and calcium lactate was added. The blend was then flavoured with hop pellets as described above and finally carbonized. This beverage was called Beverage B.

[0449] The two beverages were compared by taste panels trained in beer evaluation. The panellists were presented with a glass of each beverage and asked to score "beer flavour attributes" for each of the two beverages on a scale from 0-5, where 0 designates absence of a specific flavour and 5 designates a very intense flavour. Finally, the panellists were asked to give a total flavour score for each beverage on a scale from 0-9, where 0 is undrinkable and 9 is excellent.

[0450] Table 10 shows the contents of fermentable sugars and organic acids in Beverage A and Beverage B. It is evident that the two beverages contain practically the same total amounts of fermentable sugars and organic acids. However, due to the natural content of tartaric, malic, acetic, citric, and succinic acid in wort, the acid compositions of the two beverages are not completely identical.

[0451] Figure 6 shows the beverage flavour profiles. Beverage A scored significantly higher than Beverage B in freshness, drinkability, aromatic, estery, floral, and balance. The perceived sweetness and acidity of Beverage A was also somewhat higher than that of Beverage B. In contrast, Beverage B scored higher in less desirable flavours such as malty, grainy, caramel, and burnt than Beverage A. The total flavour score for Beverage A was 7,1, which is significantly higher than the total flavour score for Beverage B, which was 5.9.

[0452] Furthermore, a taste panel of 19 were presented with the beverages in a triangular test. In triangular tests each panelist is presented with 3 samples, two of which are identical. All 19 were able to identify, which samples were identical, thus demonstrating a significant difference in taste of beverage A and B. 13 of the panelist identified beverage A as the preferred beverage and 1 found beverage A and B equally good.

Table 10. Composition of Beverage A and Beverage B.

		Beverage A	Beverage B
<i>Fermentable sugars (g/L)</i>	Glucose	36,9	38,9
	Fructose	0,6	0,7
	Sucrose	2,7	1,2
	Maltose	3,0	1,8
	Maltotriose	0	0
	<i>Total fermentable sugars</i>	<u>43,3</u>	<u>42,6</u>
<i>Organic acids (g/L)</i>	Tartaric	0	0,2
	Malic	0	0,1
	Lactic	6,7	5,8
	Acetic	0	0,3

		Beverage A	Beverage B
	Citric	0	0,1
	Succinic	0	0,4
	<i>Total organic acids</i>	<u>6,7</u>	<u>6,9</u>
	<i>pH</i>	<u>4,35</u>	<u>4,35</u>

Example 10

[0453] 50 L glucose wort prepared essentially as described in Example 4 was transferred to the tank of a REED equipment (corresponding to 4 on figure 4). The REED equipment was set up with Ionics AR103/Nafion N117 AX-membranes and Nafion N117/Fumatech FAB CX-membranes. The AX-REED and the CX-REED membrane stack were connected in parallel. An enzyme preparation containing both glucose oxidase and catalase activity was added to the glucose wort at a dose of 2 g/L. In order to oxygenate the wort, oxygen enriched air (approx. 52% oxygen) was bubbled through the wort by means of a gas diffuser attached to a tube. The tube with the diffuser was inserted into the tank via a venting hole at the top of the tank and the length of the tube was adjusted in order to position the diffuser near the bottom of the tank. Throughout the enzymatic treatment, oxygen was supplied via the gas diffuser.

[0454] The experiment was repeated twice - the individual experiments are herein named trial 48 and trial 49. In trial 48, the enzymatic treatment and REED treatment was run for 21.5 hours at 30°C. In trial 49, the enzymatic treatment and REED treatment was run for 23 hours at 13°C. Calcium lactate was added at the start of trial 49 in order to increase the calcium content in the REED liquid. A high calcium content is desirable from a nutritional point of view.

[0455] Vitamin content and mineral content was determined in the glucose wort and in the product obtained after the enzymatic treatment. The results are shown in Tables 11 and 12. The Ca-recovery was not calculated for trial no. 49, because Ca-lactate was deliberately added at the start of the process.

Table 11 B1 and B2 vitamins in trial 48

	B1, thiamine	B2, riboflavin
Start of process (glucose wort)	392 µg/L	217 µg/L
End of process (liquid after enzymatic treatment)	294 µg/L	171 µg/L
Recovery	75%	79%

Table 12

	recovery, %		
trial no.	Ca	Mg	Fe
48	122	89	151

trial no.	recovery, %		
	Ca	Mg	Fe
49	-	99	129

Example 11

[0456] 210 kg Spanish lemons (cultivar Verna) were cut in halves and squeezed to obtain lemon juice. Pulp and pits were removed by filtration through fine-meshed sieves, and finer sediments were then removed by centrifugation at 3000 x G for 10 minutes. A total of about 54 L juice was obtained. The taste of this juice was very acidic; the citric acid content was 57 g/L, pH was 2,8, and conductivity was 4,8 mS/cm². In order to improve the taste of the juice, two trials were carried out where most of the citric acid content was removed by treatment in a REED equipment. The REED equipment used for this purpose was set up with Ionics AR103/Nafion N117 AX-membranes and Nafion N117/Fumatech FAB CX-membranes. In one trial (trial 59), the lemon juice was first subjected to AX-REED only followed by CX-REED only. Thus, the AX-REED and the CX-REED were performed in series one after the other. In another trial (trial 60), the lemon juice was first subjected to AX-REED, and subsequently the AX-REED and CX-REED were then run in parallel.

[0457] In trial 59, 25 L juice was transferred to a tank connected with the REED rig, and the AX-REED was started. Citrate was gradually removed from the liquid, and pH rose. After about 6.25 hours, pH was 4.5. An aliquot of the liquid removed for tasting seemed somewhat acidic, but rather bland and somewhat salty. The AX-REED was now stopped, and the CX-REED started. During this step, both pH and conductivity decreased. After approx. 0.75 hours, pH was 3.5, and the conductivity was 3,5 mS/cm². The CX-process was then stopped, and the liquid was collected for tasting and analyses. In order to arrive at comparable pH and conductivity in both trial 59 and 60, then it was required that the AX-REED was performed until a higher pH was reached than the desired end pH, because during CX-REED the pH falls. The pH profile during the experiment is shown in figure 7A.

[0458] In trial 60, another 25 L juice was transferred to a tank connected with the REED rig, and the AX-REED was started. As in trial 59, citrate was gradually removed from the liquid, and pH rose. After approx. 5.5 hours, pH was 3.5. An aliquot of the liquid removed for tasting still seemed quite acidic and somewhat salty. The AX-REED was continued, but the CX-REED was now also started. The two processes were allowed to run in parallel for approx. 2.5 hours. Throughout this period, citrate was still gradually removed, and the conductivity was gradually decreased to 3.5 mS/cm², but pH was kept at approx. 3,5 throughout this step. Both the AX REED and the CX REED were then stopped, and the liquid was collected for tasting and analyses. In this process the AX-REED is allowed to run until the desired end pH is achieved, and then this pH is maintained by running AX-REED and CX-REED in parallel. The pH profile is

shown in figure 7B.

[0459] Vitamin C content in the starting juice and in the final products was determined. Limonene, the dominating aroma compound in citrus fruits, was also determined. Analytical data for the lemon juice before processing and the final juices obtained from trial 59 and trial 60 are shown in table 13.

[0460] The juices obtained from the two trials were evaluated by trained tasters in triangular tests. In these tests, each of 19 panellists was presented with a sample set composed of two samples of one of the juices together with one sample of the other. The panellists were asked to identify the two identical samples in the set and then to select their preference, either the two identical samples or the single sample. Results are shown in table 14. Preference data are only shown for the 15 panellists who had the correct combination of samples. There was a significant difference between the juices, and clear and statistical significant preference for juice from trial 60, where the AX-REED and the CX-REED were run in parallel during the last part of the process, and pH was kept constant at approx. 3.5.

Table 13. Analytical data for fresh lemon juice and juices after REED treatment.

	Freshly squeezed lemon juice	Juice from trial 59	Juice from trial 60
Citric acid	57 g/L	9 g/L	9 g/L
pH	2,8	3,5	3,5
Conductivity (mS/cm ²)	4,8	3,5	3,5
Vitamin C,	380 mg/L	140 mg/L	180 mg/L
Vitamin C, % recovery		37%	47%
Limonene	370 mg/L	208 mg/L	351 mg/L
Limonene, % recovery		56%	95%

Table 14. Triangular tests performed on REED-treated lemon juices from trial 1 and trial 2.

	Number of panellists
Total number	19
Correct combination	15
Incorrect combination	4
Preference for juice from trial 59	4
Preference for juice from trial 60	10
No preference	1

Example 12

[0461] Apple juice prepared from concentrate was purchased at a local grocery store. The apple juice was subjected to a REED fermentation essentially as described in Example 4; however, with the following amendments: 52 L apple juice was used instead of glucose wort, 170 g 46% potassium hydroxide was added at the start of fermentation in order to increase pH to 4.5, fermentation temperature was 30°C, and the REED fermentation was terminated after 45 hours. The resulting liquid is also referred to as REED liquid.

[0462] The sugar content in apple juice and in the REED liquid were determined as described in Example 3 and the results are shown in Table 15. Results for organic acids in the REED liquid, determined as described in Example 3, are also included in Table 15. According to Eisele and Drake (2005), Journal of Food Composition and Analysis 18: 213-221, the malic acid content in apple juice is in the range 1,9-17,4 g/L, with an average of 8,5 g/L; the contents of other organic acids are typically < 1 g/L. It is evident that the REED liquid is reduced in sugar content, and the contents of organic acids differ from typical contents in apple juice.

Table 15

	Apple juice	REED liquid
Glucose, g/L	23,8	11,5
Fructose, g/L	58,7	35,4
Sucrose, g/L	12,7	10,9
Total fermentable sugars, g/L	95,2	57,8
Malic acid, g/L	n.d	0,6
Lactic acid, g/L	n.d	2,1
pH	3,4	3,8

SEQUENCE LISTING

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<120> Method for producing beverages by acid removal

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<170> PatentIn version 3.5

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Tyr Arg Ala Ile Lys Glu Trp Ile Gly Asn Gly Gly Val Thr Val Ser
500 505 510

Ser Ile Ser Leu Pro Phe Phe Lys Lys Phe Asp Ser Ser Ala Thr Ser
515 520 525

Gly Lys Lys Tyr Thr Val Gly Thr Ser Asp Phe Asn Asn Leu Ala Gln
530 535 540

Asn Ile Ala Leu Ala Ala Asp Arg Phe Leu Ser Thr Val Gln Leu His
545 550 555 560

Ala His Asn Asn Gly Ser Leu Ala Glu Glu Phe Asp Arg Thr Thr Gly
565 570 575

Leu Ser Thr Gly Ala Arg Asp Leu Thr Trp Ser His Ala Ser Leu Ile
580 585 590

Thr Ala Ser Tyr Ala Lys Ala Gly Ala Pro Ala Ala
595 600

<210> 4

<211> 484

<212> PRT

<213> Aspergillus niger

<400> 4

Leu Ser Ala Ala Ser Trp Arg Thr Gln Ser Ile Tyr Phe Leu Leu Thr
1 5 10 15

Asp Arg Phe Gly Arg Thr Asp Asn Ser Thr Thr Ala Thr Cys Asn Thr
20 25 30

Gly Asn Glu Ile Tyr Cys Gly Gly Ser Trp Gln Gly Ile Ile Asp His
35 40 45

Leu Asp Tyr Ile Glu Gly Met Gly Phe Thr Ala Ile Trp Ile Ser Pro
50 55 60

Ile Thr Glu Gln Leu Pro Gln Asp Thr Ala Asp Gly Glu Ala Tyr His
65 70 75 80

Gly Tyr Trp Gln Gln Lys Ile Tyr Asp Val Asn Ser Asn Phe Gly Thr

				85					90					95	
Ala	Asp	Asn	Leu	Lys	Ser	Leu	Ser	Asp	Ala	Leu	His	Ala	Arg	Gly	Met
			100					105					110		
Tyr	Leu	Met	Val	Asp	Val	Val	Pro	Asp	His	Met	Gly	Tyr	Ala	Gly	Asn
		115					120					125			
Gly	Asn	Asp	Val	Asp	Tyr	Ser	Val	Phe	Asp	Pro	Phe	Asp	Ser	Ser	Ser
	130					135					140				
Tyr	Phe	His	Pro	Tyr	Cys	Leu	Ile	Thr	Asp	Trp	Asp	Asn	Leu	Thr	Met
145					150					155					160
Val	Glu	Asp	Cys	Trp	Glu	Gly	Asp	Thr	Ile	Val	Ser	Leu	Pro	Asp	Leu
				165					170					175	
Asp	Thr	Thr	Glu	Thr	Ala	Val	Arg	Thr	Ile	Trp	Tyr	Asp	Trp	Val	Ala
			180					185					190		
Asp	Leu	Val	Ser	Asn	Tyr	Ser	Val	Asp	Gly	Leu	Arg	Ile	Asp	Ser	Val
		195					200					205			
Leu	Glu	Val	Gln	Pro	Asp	Phe	Phe	Pro	Gly	Tyr	Asn	Lys	Ala	Ser	Gly
	210					215					220				
Val	Tyr	Cys	Val	Gly	Glu	Ile	Asp	Asn	Gly	Asn	Pro	Ala	Ser	Asp	Cys
225					230					235					240
Pro	Tyr	Gln	Lys	Val	Leu	Asp	Gly	Val	Leu	Asn	Tyr	Pro	Ile	Tyr	Trp
				245					250					255	
Gln	Leu	Leu	Tyr	Ala	Phe	Glu	Ser	Ser	Ser	Gly	Ser	Ile	Ser	Asn	Leu
			260					265					270		
Tyr	Asn	Met	Ile	Lys	Ser	Val	Ala	Ser	Asp	Cys	Ser	Asp	Pro	Thr	Leu
		275					280					285			
Leu	Gly	Asn	Phe	Ile	Glu	Asn	His	Asp	Asn	Pro	Arg	Phe	Ala	Lys	Tyr
	290					295					300				
Thr	Ser	Asp	Tyr	Ser	Gln	Ala	Lys	Asn	Val	Leu	Ser	Tyr	Ile	Phe	Leu
305					310					315					320
Ser	Asp	Gly	Ile	Pro	Ile	Val	Tyr	Ala	Gly	Glu	Glu	Gln	His	Tyr	Ala
				325					330					335	
Gly	Gly	Lys	Val	Pro	Tyr	Asn	Arg	Glu	Ala	Thr	Trp	Leu	Ser	Gly	Tyr
			340					345					350		
Asp	Thr	Ser	Ala	Glu	Leu	Tyr	Thr	Trp	Ile	Ala	Thr	Thr	Asn	Ala	Ile
		355					360					365			
Arg	Lys	Leu	Ala	Ile	Ala	Ala	Asp	Ser	Ala	Tyr	Ile	Thr	Tyr	Ala	Asn
	370					375					380				

Asp Ala Phe Tyr Thr Asp Ser Asn Thr Ile Ala Met Ala Lys Gly Thr
385 390 395 400

Ser Gly Ser Gln Val Ile Thr Val Leu Ser Asn Lys Gly Ser Ser Gly
405 410 415

Ser Ser Tyr Thr Leu Thr Leu Ser Gly Ser Gly Tyr Thr Ser Gly Thr
420 425 430

Lys Leu Ile Glu Ala Tyr Thr Cys Thr Ser Val Thr Val Asp Ser Ser
435 440 445

Gly Asp Ile Pro Val Pro Met Ala Ser Gly Leu Pro Arg Val Leu Leu
450 455 460

Pro Ala Ser Val Val Asp Ser Ser Ser Leu Cys Gly Gly Ser Gly Arg
465 470 475 480

Leu Tyr Val Glu

<210> 5

<211> 499

<212> PRT

<213> Aspergillus oryzae

<400> 5

Met Met Val Ala Trp Trp Ser Leu Phe Leu Tyr Gly Leu Gln Val Ala
1 5 10 15

Ala Pro Ala Leu Ala Ala Thr Pro Ala Asp Trp Arg Ser Gln Ser Ile
20 25 30

Tyr Phe Leu Leu Thr Asp Arg Phe Ala Arg Thr Asp Gly Ser Thr Thr
35 40 45

Ala Thr Cys Asn Thr Ala Asp Arg Lys Tyr Cys Gly Gly Thr Trp Gln
50 55 60

Gly Ile Ile Asp Lys Leu Asp Tyr Ile Gln Gly Met Gly Phe Thr Ala
65 70 75 80

Ile Trp Ile Thr Pro Val Thr Ala Gln Leu Pro Gln Thr Thr Ala Tyr
85 90 95

Gly Asp Ala Tyr His Gly Tyr Trp Gln Gln Asp Ile Tyr Ser Leu Asn
100 105 110

Glu Asn Tyr Gly Thr Ala Asp Asp Leu Lys Ala Leu Ser Ser Ala Leu
115 120 125

His Glu Arg Gly Met Tyr Leu Met Val Asp Val Val Ala Asn His Met
130 135 140

Gly Tyr Asp Gly Ala Gly Ser Ser Val Asp Tyr Ser Val Phe Lys Pro
145 150 155 160

Phe Ser Ser Gln Asp Tyr Phe His Pro Phe Cys Leu Ile Gln Asn Tyr
 165 170 175

Glu Asp Gln Thr Gln Val Glu Asp Cys Trp Leu Gly Asp Asn Thr Val
 180 185 190

Ser Leu Pro Asp Leu Asp Thr Thr Lys Asp Val Val Lys Asn Glu Trp
 195 200 205

Tyr Asp Trp Val Gly Ser Leu Val Ser Asn Tyr Ser Ile Asp Gly Leu
 210 215 220

Arg Ile Asp Thr Val Lys His Val Gln Lys Asp Phe Trp Pro Gly Tyr
 225 230 235 240

Asn Lys Ala Ala Gly Val Tyr Cys Ile Gly Glu Val Leu Asp Gly Asp
 245 250 255

Pro Ala Tyr Thr Cys Pro Tyr Gln Asn Val Met Asp Gly Val Leu Asn
 260 265 270

Tyr Pro Ile Tyr Tyr Pro Leu Leu Asn Ala Phe Lys Ser Thr Ser Gly
 275 280 285

Ser Met Asp Asp Leu Tyr Asn Met Ile Asn Thr Val Lys Ser Asp Cys
 290 295 300

Pro Asp Ser Thr Leu Leu Gly Thr Phe Val Glu Asn His Asp Asn Pro
 305 310 315 320

Arg Phe Ala Ser Tyr Thr Asn Asp Ile Ala Leu Ala Lys Asn Val Ala
 325 330 335

Ala Phe Ile Ile Leu Asn Asp Gly Ile Pro Ile Ile Tyr Ala Gly Gln
 340 345 350

Glu Gln His Tyr Ala Gly Gly Asn Asp Pro Ala Asn Arg Glu Ala Thr
 355 360 365

Trp Leu Ser Gly Tyr Pro Thr Asp Ser Glu Leu Tyr Lys Leu Ile Ala
 370 375 380

Ser Ala Asn Ala Ile Arg Asn Tyr Ala Ile Ser Lys Asp Thr Gly Phe
 385 390 395 400

Val Thr Tyr Lys Asn Trp Pro Ile Tyr Lys Asp Asp Thr Thr Ile Ala
 405 410 415

Met Arg Lys Gly Thr Asp Gly Ser Gln Ile Val Thr Ile Leu Ser Asn
 420 425 430

Lys Gly Ala Ser Gly Asp Ser Tyr Thr Leu Ser Leu Ser Gly Ala Gly
 435 440 445

Tyr Thr Ala Gly Gln Gln Leu Thr Glu Val Ile Gly Cys Thr Thr Val
 450 455 460

Thr Val Gly Ser Asp Gly Asn Val Pro Val Pro Met Ala Gly Gly Leu
465 470 475 480

Pro Arg Val Leu Tyr Pro Thr Glu Lys Leu Ala Gly Ser Lys Ile Cys
485 490 495

Ser Ser Ser

<210> 6

<211> 462

<212> PRT

<213> Rhizopus oryzae

<400> 6

Met Lys Ser Phe Leu Ser Leu Leu Cys Ser Val Phe Leu Leu Pro Leu
1 5 10 15

Val Val Gln Ser Val Pro Val Ile Lys Arg Ala Ser Ala Ser Asp Trp
20 25 30

Glu Asn Arg Val Ile Tyr Gln Leu Leu Thr Asp Arg Phe Ala Lys Ser
35 40 45

Thr Asp Asp Thr Asn Gly Cys Ser Asn Leu Ser Asp Tyr Cys Gly Gly
50 55 60

Thr Phe Gln Gly Ile Ile Asn His Leu Asp Tyr Ile Ala Gly Met Gly
65 70 75 80

Phe Asp Ala Ile Trp Ile Ser Pro Ile Pro Lys Asn Val Asn Gly Gly
85 90 95

Tyr His Gly Tyr Trp Ala Ser Asp Phe Ser Gln Ile Asn Glu His Phe
100 105 110

Gly Thr Ala Asp Asp Leu Lys Lys Leu Val Ala Ala Ala His Ala Lys
115 120 125

Asn Met Tyr Val Met Leu Asp Val Val Ala Asn His Ala Gly Thr Pro
130 135 140

Ser Ser Gly Gly Asp Tyr Ser Gly Tyr Thr Phe Gly Gln Ser Ser Glu
145 150 155 160

Tyr His Arg Ala Cys Asp Ile Asn Tyr Asn Asp Gln Asn Ser Ile Glu
165 170 175

Gln Cys Trp Ile Ser Gly Leu Pro Asp Ile Asn Thr Glu Asp Ser Ala
180 185 190

Ile Val Ser Lys Leu Asn Ser Ile Val Ser Gly Trp Val Ser Asp Tyr
195 200 205

Gly Phe Asp Gly Leu Arg Ile Asp Thr Val Lys His Val Arg Lys Asp
210 215 220

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      210      215      220
Phe Trp Asp Gly Tyr Val Ser Ala Ala Gly Val Phe Ala Thr Gly Glu
225                230                235                240

Val Leu Ser Gly Asp Val Ser Tyr Val Ser Pro Tyr Gln Gln His Val
                245                250                255

Pro Ser Leu Ile Asn Tyr Pro Leu Tyr Tyr Pro Val Tyr Asp Val Phe
                260                265                270

Thr Lys Ser Arg Thr Met Ser Arg Leu Ser Ser Gly Phe Ser Asp Ile
                275                280                285

Lys Asn Gly Asn Phe Lys Asn Ile Asp Val Leu Val Asn Phe Ile Asp
                290                295                300

Asn His Asp Gln Pro Arg Leu Leu Ser Lys Ala Asp Gln Ser Leu Val
305                310                315                320

Lys Asn Ala Leu Ala Tyr Ser Phe Met Val Gln Gly Ile Pro Val Leu
                325                330                335

Tyr Tyr Gly Thr Glu Gln Ser Phe Lys Gly Gly Asn Asp Pro Asn Asn
                340                345                350

Arg Glu Val Leu Trp Thr Thr Gly Tyr Ser Thr Thr Ser Asp Met Tyr
                355                360                365

Lys Phe Val Thr Thr Leu Val Lys Ala Arg Lys Gly Ser Asn Ser Thr
                370                375                380

Val Asn Met Gly Ile Ala Gln Thr Asp Asn Val Tyr Val Phe Gln Arg
385                390                395                400

Gly Gly Ser Leu Val Val Val Asn Asn Tyr Gly Gln Gly Ser Thr Asn
                405                410                415

Thr Ile Thr Val Lys Ala Gly Ser Phe Ser Asn Gly Asp Thr Leu Thr
                420                425                430

Asp Val Phe Ser Asn Lys Ser Val Thr Val Gln Asn Asn Gln Ile Thr
                435                440                445

Phe Gln Leu Gln Asn Gly Asn Pro Ala Ile Phe Gln Lys Asn
                450                455                460

<210> 7
<211> 718
<212> PRT
<213> Bacillus subtilis

<400> 7
Met Val Ser Ile Arg Arg Ser Phe Glu Ala Tyr Val Asp Asp Met Asn
1                5                10                15

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Ile Ile Thr Val Leu Ile Pro Ala Glu Gln Lys Glu Ile Met Thr Pro
      20                      25                      30

Pro Phe Arg Leu Glu Thr Glu Ile Thr Asp Phe Pro Leu Ala Val Arg
      35                      40                      45

Glu Glu Tyr Ser Leu Glu Ala Lys Tyr Lys Tyr Val Cys Val Ser Asp
      50                      55                      60

His Pro Val Thr Phe Gly Lys Ile His Cys Val Arg Ala Ser Ser Gly
      65                      70                      75                      80

His Lys Thr Asp Leu Gln Ile Gly Ala Val Ile Arg Thr Ala Ala Phe
      85                      90                      95

Asp Asp Glu Phe Tyr Tyr Asp Gly Glu Leu Gly Ala Val Tyr Thr Ala
      100                      105                      110

Asp His Thr Val Phe Lys Val Trp Ala Pro Ala Ala Thr Ser Ala Ala
      115                      120                      125

Val Lys Leu Ser His Pro Asn Lys Ser Gly Arg Thr Phe Gln Met Thr
      130                      135                      140

Arg Leu Glu Lys Gly Val Tyr Ala Val Thr Val Thr Gly Asp Leu His
      145                      150                      155                      160

Gly Tyr Glu Tyr Leu Phe Cys Ile Cys Asn Asn Ser Glu Trp Met Glu
      165                      170                      175

Thr Val Asp Gln Tyr Ala Lys Ala Val Thr Val Asn Gly Glu Lys Gly
      180                      185                      190

Val Val Leu Arg Pro Asp Gln Met Lys Trp Thr Ala Pro Leu Lys Pro
      195                      200                      205

Phe Ser His Pro Val Asp Ala Val Ile Tyr Glu Thr His Leu Arg Asp
      210                      215                      220

Phe Ser Ile His Glu Asn Ser Gly Met Ile Asn Lys Gly Lys Tyr Leu
      225                      230                      235                      240

Ala Leu Thr Glu Thr Asp Thr Gln Thr Ala Asn Gly Ser Ser Ser Gly
      245                      250                      255

Leu Ala Tyr Val Lys Glu Leu Gly Val Thr His Val Glu Leu Leu Pro
      260                      265                      270

Val Asn Asp Phe Ala Gly Val Asp Glu Glu Lys Pro Leu Asp Ala Tyr
      275                      280                      285

Asn Trp Gly Tyr Asn Pro Leu His Phe Phe Ala Pro Glu Gly Ser Tyr
      290                      295                      300

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Ala	Ser	Asn	Pro	His	Asp	Pro	Gln	Thr	Arg	Lys	Thr	Glu	Leu	Lys	Gln	305	310	315	320
Met	Ile	Asn	Thr	Leu	His	Gln	His	Gly	Leu	Arg	Val	Ile	Leu	Asp	Val	325	330	335	
Val	Phe	Asn	His	Val	Tyr	Lys	Arg	Glu	Asn	Ser	Pro	Phe	Glu	Lys	Thr	340	345	350	
Val	Pro	Gly	Tyr	Phe	Phe	Arg	His	Asp	Glu	Cys	Gly	Met	Pro	Ser	Asn	355	360	365	
Gly	Thr	Gly	Val	Gly	Asn	Asp	Ile	Ala	Ser	Glu	Arg	Arg	Met	Ala	Arg	370	375	380	
Lys	Phe	Ile	Ala	Asp	Cys	Val	Val	Tyr	Trp	Leu	Glu	Glu	Tyr	Asn	Val	385	390	395	400
Asp	Gly	Phe	Arg	Phe	Asp	Leu	Leu	Gly	Ile	Leu	Asp	Ile	Asp	Thr	Val	405	410	415	
Leu	Tyr	Met	Lys	Glu	Lys	Ala	Thr	Lys	Ala	Lys	Pro	Gly	Ile	Leu	Leu	420	425	430	
Phe	Gly	Glu	Gly	Trp	Asp	Leu	Ala	Thr	Pro	Leu	Pro	His	Glu	Gln	Lys	435	440	445	
Ala	Ala	Leu	Ala	Asn	Ala	Pro	Arg	Met	Pro	Gly	Ile	Gly	Phe	Phe	Asn	450	455	460	
Asp	Met	Phe	Arg	Asp	Ala	Val	Lys	Gly	Asn	Thr	Phe	His	Leu	Lys	Ala	465	470	475	480
Thr	Gly	Phe	Ala	Leu	Gly	Asn	Gly	Glu	Ser	Ala	Gln	Ala	Val	Met	His	485	490	495	
Gly	Ile	Ala	Gly	Ser	Ser	Gly	Trp	Lys	Ala	Leu	Ala	Pro	Ile	Val	Pro	500	505	510	
Glu	Pro	Ser	Gln	Ser	Ile	Asn	Tyr	Val	Glu	Ser	His	Asp	Asn	His	Thr	515	520	525	
Phe	Trp	Asp	Lys	Met	Ser	Phe	Ala	Leu	Pro	Gln	Glu	Asn	Asp	Ser	Arg	530	535	540	
Lys	Arg	Ser	Arg	Gln	Arg	Leu	Ala	Ala	Ala	Ile	Ile	Leu	Leu	Ala	Gln	545	550	555	560
Gly	Val	Pro	Phe	Ile	His	Ser	Gly	Gln	Glu	Phe	Phe	Arg	Thr	Lys	Gln	565	570	575	
Gly	Val	Glu	Asn	Ser	Tyr	Gln	Ser	Ser	Asp	Ser	Ile	Asn	Gln	Leu	Asp	580	585	590	
Trp	Asp	Arg	Arg	Glu	Thr	Phe	Lys	Glu	Asp	Val	His	Tyr	Ile	Arg	Arg	595	600	605	

Leu Ile Ser Leu Arg Lys Ala His Pro Ala Phe Arg Leu Arg Ser Ala
610 615 620

Ala Asp Ile Gln Arg His Leu Glu Cys Leu Thr Leu Lys Glu His Leu
625 630 635 640

Ile Ala Tyr Arg Leu Tyr Asp Leu Asp Glu Val Asp Glu Trp Lys Asp
645 650 655

Ile Ile Val Ile His His Ala Ser Pro Asp Ser Val Glu Trp Arg Leu
660 665 670

Pro Asn Asp Ile Pro Tyr Arg Leu Leu Cys Asp Pro Ser Gly Phe Gln
675 680 685

Glu Asp Pro Thr Glu Ile Lys Lys Thr Val Ala Val Asn Gly Ile Gly
690 695 700

Thr Val Ile Leu Tyr Leu Ala Ser Asp Leu Lys Ser Phe Ala
705 710 715

<210> 8

<211> 850

<212> PRT

<213> Bacillus cereus (strain ZK / E33L)

<400> 8

Met Thr Lys Arg Leu Ile Asn Lys Ser Val Leu Leu Leu Thr Ile Ile
1 5 10 15

Val Met Leu Ser Ser Val Tyr Ser Phe Gln Asn Val Lys Ala Val Ser
20 25 30

Asn Ser Lys Thr Thr Glu Val Ile Ile His Tyr Lys Glu Gln Ser Gly
35 40 45

Asn Thr Lys Asp Trp Asn Leu Trp Ile Trp Gly Glu Asn Ser Asn Gly
50 55 60

Lys Ser Tyr Glu Phe Thr Gly Glu Asp Glu Phe Gly Lys Tyr Ala Lys
65 70 75 80

Ile Asn Ile Asp Gly Asp Tyr Asn Arg Leu Gly Phe Ile Ile Arg Thr
85 90 95

Ser Glu Trp Glu Lys Asp Gly Gly Asp Arg Trp Ile Glu Asn Ile Lys
100 105 110

Asp Gly Arg Ala Glu Val Trp Ile Leu Ser Gly Asp Glu Lys Val Tyr
115 120 125

Asn Ser Lys Pro Ser Ser Asp Leu Ser Ile Gln Lys Ala Thr Ile Asp
130 135 140

Ser Phe His Glu Ile Thr Val Thr Thr Asn Val Pro Phe His Ile Lys
145 150 155 160

Glu Lys Lys Ile Glu Met Glu Gly Ile Lys Ile Lys Ser Ile Ser Pro
165 170 175

Tyr Asp Ile Asn Ser Gly Asp Ile Thr Asn Lys Val Lys Ile Ile Thr
180 185 190

Asp Gln Lys Ile Asp Leu Lys Gln Thr Tyr Lys Val Lys Ile Glu Asn
195 200 205

Leu Ala Asp Thr Asn Thr Glu Ile Gly Lys Val Ile Arg Thr Glu Glu
210 215 220

Phe Asp Lys Leu Phe Tyr Tyr Gly Gly Asn Asp Leu Gly Asn Ile Tyr
225 230 235 240

Thr Pro Gln His Thr Lys Phe Arg Val Trp Ala Pro Thr Ala Ser Glu
245 250 255

Ala Lys Leu Val Thr Tyr Lys Lys Trp Asn Asp Lys Ile Gly Thr Glu
260 265 270

Ile Asn Met Gln Gln Gly Glu Lys Gly Thr Trp Lys Ala Glu Leu Lys
275 280 285

Gly Asn Gln Lys Gly Leu Tyr Tyr Thr Tyr Lys Val Lys Ile Gly Asp
290 295 300

Lys Trp Thr Glu Ala Val Asp Pro Tyr Ala Arg Ala Ala Ser Val Asn
305 310 315 320

Gly Asp Lys Gly Ala Val Val Asp Leu Glu Glu Thr Asn Pro Lys Arg
325 330 335

Trp Asn Thr Asn Lys Lys Pro Lys Leu Lys Asn Pro Glu Asp Ala Ile
340 345 350

Ile Tyr Glu Leu His Val Arg Asp Leu Ser Ile Gln Pro Glu Ser Gly
355 360 365

Ile Lys Gln Lys Gly Lys Tyr Leu Gly Val Thr Glu Lys Gly Thr Lys
370 375 380

Gly Pro Glu Gly Val Lys Thr Gly Leu Asp His Met Lys Asp Leu Gly
385 390 395 400

Val Thr His Val Gln Leu Leu Pro Ile Phe Asp Tyr Ala Ser Val Asn
405 410 415

Glu Glu Lys Val Asn Glu Pro Gln Tyr Asn Trp Gly Tyr Asp Pro Lys
420 425 430

Asn Phe Asn Val Pro Glu Gly Ser Tyr Ser Thr Asn Pro Tyr Glu Pro
435 440 445

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Thr Val Arg Ile Thr Glu Leu Lys Gln Met Ile Gln Thr Leu His Asp
450                               455                               460

Asn Asn Leu Arg Val Val Met Asp Val Val Tyr Asn His Met Tyr Asn
465                               470                               475                               480

Ala Ala Glu Ser Asn Phe His Lys Leu Val Pro Gly Tyr Tyr Tyr Arg
485                               490                               495

Tyr Asn Glu Asp Gly Thr Phe Ala Asn Gly Thr Gly Val Gly Asn Asp
500                               505                               510

Thr Ala Ser Glu Arg Lys Met Met Arg Lys Phe Met Ile Asp Ser Val
515                               520                               525

Thr Tyr Trp Ala Lys Glu Tyr Asn Leu Asp Gly Phe Arg Phe Asp Leu
530                               535                               540

Met Gly Ile His Asp Tyr Glu Thr Met Asn Glu Ile Arg Lys Ala Val
545                               550                               555                               560

Asn Gln Ile Asp Pro Ser Ile Ile Leu His Gly Glu Gly Trp Asp Leu
565                               570                               575

Asn Thr Pro Leu Ala Ala Glu Leu Lys Ala Asn Gln Lys Asn Ala Glu
580                               585                               590

Lys Met Lys Gly Ile Ala His Phe Asn Asp Asn Ile Arg Asp Gly Leu
595                               600                               605

Lys Gly Ser Val Phe Glu Glu Lys Glu Asn Gly Phe Val Asn Gly Lys
610                               615                               620

Glu Asn Met Glu Asp Arg Ile Lys Lys Gly Ile Thr Ala Gly Ile Asp
625                               630                               635                               640

Tyr Asp Thr Asn Ser Ser Thr Tyr Leu Asp Pro Glu Gln Val Leu Thr
645                               650                               655

Tyr Val Glu Ala His Asp Asn His Thr Leu Trp Asp Lys Leu Glu Leu
660                               665                               670

Thr Asn Pro Gly Asp Ser Glu Glu Ala Arg Lys Gln Met His Lys Leu
675                               680                               685

Ser Ser Ser Ile Leu Leu Thr Ser Gln Gly Ile Pro Phe Leu His Ala
690                               695                               700

Gly Gln Glu Phe Met Arg Thr Lys Tyr Gly Asp His Asn Ser Tyr Lys
705                               710                               715                               720

Ser Pro Asp Ser Ile Asn Gln Met Asp Trp Leu Arg Arg Ala Ala Phe
725                               730                               735

Asn Asn Glu Val Asp Tyr Met Lys Gly Leu Ile Glu Leu Arg Lys Lys
740                               745                               750

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Tyr Pro Ala Phe Arg Met Thr Ser Ala Glu Gln Ile Lys Thr His Val
755 760 765

Ser Phe Ile Asp Ala Pro Lys Asn Thr Val Ala Tyr Thr Ile Glu Gly
770 775 780

Asn Lys Asn Glu Tyr Phe Thr Val Ala His Asn Ala Asn Arg Glu Ser
785 790 795 800

Val Glu Ile Ser Leu Pro Ser Lys Gly Pro Trp Lys Val Leu Val Asp
805 810 815

Gly Lys Gln Ala Gly Ser Lys Pro Leu Tyr Val Val His Asp Asn Lys
820 825 830

Ile Lys Val Pro Ala Leu Ser Ser Leu Val Leu Lys Thr Glu Lys Pro
835 840 845

Ile Lys
850

<210> 9

<211> 589

<212> PRT

<213> Lactobacillus acidophilus (strain ATCC 700396 / NCK56 / N2 / NCFM)

<400> 9

Met Lys Ile Thr Tyr Asp Ser Trp Gln Glu Gln Tyr Lys Asn Pro Phe
1 5 10 15

Gly Ala Val Lys Ala Gly Asn Thr Val Lys Trp Ser Ile Lys Ile Asp
20 25 30

Gln Val Ile Gln Gly Ala Val Leu Trp Leu Thr Lys Ser Arg Glu Thr
35 40 45

Pro Val Ala Tyr Pro Met Asn Tyr Asp Glu Gln Thr Lys Met Tyr Thr
50 55 60

Thr Gln Val Lys Ile Gly Thr Ser Gly Leu Tyr Asn Tyr Tyr Phe Ala

65 70 75 80

Leu Gln Gln Asn Asn Gln Ile Val Tyr Ile Asp Gln Gly Leu Phe Gly
85 90 95

Lys Gly His Val Thr Lys Ser Asp His Asp Leu Arg Gln Phe Gln Leu
100 105 110

Thr Cys Tyr Asp Ile Ala Thr Pro Arg Ile Asp Trp Tyr Gln Lys Gly
115 120 125

Ile Val Tyr Gln Ile Phe Pro Asp Arg Phe Ala Asn Gly Asn Pro Tyr
130 135 140

130	135	140
Glu Glu Val Ile Gly Lys Lys Arg Asn Ser Phe Ile Tyr Ala Thr Lys 145	150	155
Glu Asp Ile Pro Tyr Tyr Ile Lys Asn Ser Glu Gly Ala Ile Val Arg 165	170	175
Trp Asp Phe Phe Gly Gly Asn Leu Thr Gly Ile Arg Lys Lys Ile Pro 180	185	190
Tyr Leu Lys Gln Leu Gly Val Thr Val Leu Tyr Leu Asn Pro Ile Phe 195	200	205
Leu Ala Lys Ser Asn His Arg Tyr Asp Thr Thr Asp Phe Met Lys Ile 210	215	220
Asp Pro Met Leu Gly Asp Glu Lys Asp Leu Ala Asp Leu Ile Arg Glu 225	230	235
Leu His Glu Asn Asn Met His Leu Ile Leu Asp Gly Val Phe Asn His 245	250	255
Val Gly Phe Asp Ser Ile Tyr Phe Gln Gly Ala Ile Thr Asp Lys Asn 260	265	270
Ser Asn Tyr Arg Ser Trp Phe Asn Phe Gln Asp Tyr Pro Asn Lys Tyr 275	280	285
Gln Ser Trp Trp Gly Val Lys Ser Leu Pro Thr Val Asn Lys Asp Asn 290	295	300
Ser Glu Tyr Gln Asn Leu Val Tyr Gly Asp His Gly Val Leu Ala Lys 305	310	315
Trp Lys Val Asp Gly Trp Arg Leu Asp Val Ala Asp Glu Leu Pro Met 325	330	335
Asp Phe Leu Arg Asn Ile Arg Asn Arg Leu Ile Lys Glu Asn Cys Pro 340	345	350
Ile Leu Ile Gly Glu Val Trp Glu Asp Ala Ser Asn Lys Phe Val Asn 355	360	365
Gly Glu Tyr Arg Thr Tyr Thr Ala Gly Asp Asn Leu Met Gly Val Met 370	375	380
Asn Tyr Pro Ile Arg Asn Phe Ile Ile Ser Leu Leu Ser Ala Gln Asp 385	390	395
Ser Thr Ile Glu Ile Glu Ala Met Asn Asp Leu Ala Leu Leu Ile Glu 405	410	415
Asn Tyr Pro Thr Asp Phe Leu His Asn Cys Leu Asn Asn Ile Gly Thr 420	425	430
His Asp Thr Val Arg Ile Lys Thr Val Leu Asn Lys Asn Asp Asn Leu		

435 440 445
 Val Met Met Ala Phe Gly Leu Leu Phe Met Met Pro Gly Val Pro Cys
 450 455 460

 Ile Tyr Tyr Gly Asp Glu Ala Gly Leu Ile Gly Lys Glu Asp Pro Asp
 465 470 475 480

 Asn Arg Arg Tyr Phe Leu Trp Gly His Glu Asp Lys Lys Leu Ile Asp
 485 490 495

 Cys Val Ser Ser Trp Thr Lys Ile Arg Lys Gln Asn Pro Val Leu Val
 500 505 510

 Asn Gly Lys Ile Gly Phe Val His Leu Ser Ala Gly Val Asn Ser Ile
 515 520 525

 Val Arg Tyr Asn Asp Gln Glu Met Ile Met Tyr Cys Val Asn Cys Thr
 530 535 540

 Asn Glu Asp Val Ile Pro Leu Arg Glu Lys Tyr Ser Phe Tyr Trp Leu
 545 550 555 560

 Pro Ser Ile Ile Ile Asp Lys Ile Lys Asp Thr Leu Asp Gln Ile Gln
 565 570 575

 Leu Lys Ala Gln Thr Asp Phe Ile Lys Lys Ile Ser Leu
 580 585

 <210> 10
 <211> 605
 <212> PRT
 <213> Aspergillus niger

 <400> 10
 Met Gln Thr Leu Leu Val Ser Ser Leu Val Val Ser Leu Ala Ala Ala
 1 5 10 15

 Leu Pro His Tyr Ile Arg Ser Asn Gly Ile Glu Ala Ser Leu Leu Thr
 20 25 30

 Asp Pro Lys Asp Val Ser Gly Arg Thr Val Asp Tyr Ile Ile Ala Gly
 35 40 45

 Gly Gly Leu Thr Gly Leu Thr Thr Ala Ala Arg Leu Thr Glu Asn Pro
 50 55 60

 Asn Ile Ser Val Leu Val Ile Glu Ser Gly Ser Tyr Glu Ser Asp Arg
 65 70 75 80

 Gly Pro Ile Ile Glu Asp Leu Asn Ala Tyr Gly Asp Ile Phe Gly Ser
 85 90 95

 Ser Val Asp His Ala Tyr Glu Thr Val Glu Leu Ala Thr Asn Asn Gln
 100 105 110

Thr Ala Leu Ile Arg Ser Gly Asn Gly Leu Gly Gly Ser Thr Leu Val
 115 120 125

Asn Gly Gly Thr Trp Thr Arg Pro His Lys Ala Gln Val Asp Ser Trp
 130 135 140

Glu Thr Val Phe Gly Asn Glu Gly Trp Asn Trp Asp Asn Val Ala Ala
 145 150 155 160

Tyr Ser Leu Gln Ala Glu Arg Ala Arg Ala Pro Asn Ala Lys Gln Ile
 165 170 175

Ala Ala Gly His Tyr Phe Asn Ala Ser Cys His Gly Val Asn Gly Thr
 180 185 190

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Asp Phe Gly Cys Gly Asp Pro His Gly Val Ser Met Phe Pro Asn Thr
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Gly Lys Val Leu Leu Ser Gln Asn Gly Thr Thr Pro Arg Ala Val Gly
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      675                      680                      685

Ala Gly Ile Asp Ser Ser Arg Ser Gly Val Tyr Thr Gly Ser Ser Glu
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705                      710                      715                      720

Arg Phe Val Asp Arg Phe Ala Leu Asp Glu
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REFERENCES CITED IN THE DESCRIPTION

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PATENTKRAV

1. Fremgangsmåde til fremstilling af en drikkevare, hvor fremgangsmåden omfatter følgende trin

- 5 a) tilvejebringelse af en startvæske omfattende mindst ét mikronæringsstof og mindst én organisk syre, hvor mikronæringsstoffet er valgt fra gruppen bestående af mineraler, vitaminer, salte og antioxidanter; og
- 10 d) fjernelse af i det mindste noget af den organiske syre fra væsken, hvor den sure ion fjernes gennem en Anion Exchange Reverse Electro-Enhanced Dialysis (AX-REED)-membranstak, hvor membranstakken indeholder
- i) mindst én celle bestående af:
- a. to anionbyttermembraner, der definerer et kammer til startvæsken; og
- b. to yderligere kamre til en dialysevæske, hvor de to yderligere kamre er placeret ved siden af kammeret til startvæsken på modstående sider, og hvor de to
- 15 yderligere kamre kan være forbundet til
- ii) et sæt endemembraner
- iii) midler til påføring af et elektrisk felt over membranstakken ved hjælp af mindst to elektroder
- iv) midler til at skifte retningen af det elektriske felt inde i membranstakken
- 20 og hvor fjernelsen involverer følgende trin
- I. indsættelse af startvæsken i kammeret til startvæsken; og
- II. indsættelse af en dialysevæske i de to yderligere kamre til dialysevæsken; og
- III. påføring af et elektrisk felt over membranstakken;
- IV. inkubation af startvæsken i kammeret, hvorved retningen af det elektriske
- 25 felt skiftes med intervaller

og

5 e) fjernelse af i det mindste en del af én kation fra startvæsken eller fra delvist AX-REED-behandlet væske, hvorved der opnås en REED-væske, hvor kationen fjernes gennem en Cation Exchange Reverse Electro-Enhanced Dialysis (CX-REED)-membranstak, hvor membranstakken indeholder

i) mindst én celle bestående af:

a. to kationbyttermembraner, der definerer et kammer til startvæsken eller den delvist AX-REED-behandlede væske; og

10 b. to yderligere kamre til en anden dialysevæske, hvor de to yderligere kamre er placeret ved siden af kammeret til startvæsken eller den delvist AX-REED-behandlede væske på modstående sider, og hvor de to yderligere kamre kan være forbundet til

ii) et sæt endemembraner

15 iii) midler til påføring af et elektrisk felt over membranstakken ved hjælp af mindst to elektroder

iv) midler til at skifte retningen af det elektriske felt inde i membranstakken

og hvor fjernelsen involverer følgende trin

I. indsættelse af startvæsken eller den delvist AX-REED-behandlede væske i kammeret til startvæsken eller den delvist AX-REED-behandlede væske; og

20 II. indsættelse af en anden dialysevæske i de to yderligere kamre til den anden dialysevæske; og

III. påføring af et elektrisk felt over membranstakken;

IV. inkubation af startvæsken eller den delvist AX-REED-behandlede væske i kammeret, hvorved retningen af det elektriske felt skiftes med intervaller,

- hvor trin d) og e) udføres i det mindste delvist samtidigt, og hvor AX-REED-membranstakken og CX-REED-membranstakken er forbundet til en tank, og AX-REED modtager startvæske fra tanken, og startvæsken returneres til tanken efter behandling i AX-REED-stakken, og CX-REED modtager startvæske eller delvist AX-REED-behandlet væske eller AX-REED-væske fra tanken, og væsken returneres til tanken efter behandling i CX-REED-stakken,
- hvor AX-REED-membranstakken er forbundet til CX-REED-membranstakken parallelt, og
- hvor mindst 65% af det mindst ene mikronæringsstof tilbageholdes i REED-væsken.
2. Fremgangsmåde ifølge krav 1, hvor
- trin a) omfatter tilvejebringelse af en startvæske omfattende mindst ét mikronæringsstof, mindst én aromaforbindelse og mindst ét sukker; og
- hvor REED-væsken indeholder mindst 65% af den mindst ene aromaforbindelse, som er til stede i startvæsken.
3. Fremgangsmåde ifølge krav 2, hvor REED-væsken omfatter mindst 80% af mindst to, fortrinsvis af mindst tre aromaforbindelser, som er til stede i startvæsken.
4. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 3, hvor startvæsken er et ekstrakt, koncentrat eller en saft af en plante eller en plantedel, der er blevet behandlet med ét eller flere enzymer, eller en frugtsaft eller en fermenteret frugtsaft.
5. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor startvæsken indeholder vitamin C, og CX-REED-væsken indeholder mindst 40%, fortrinsvis mindst 45% af det vitamin C, der er indeholdt i startvæsken.
6. Fremgangsmåde ifølge et hvilket som helst af kravene 2 til 5, hvor mindst én aromaforbindelse er en ester, en terpen eller et terpenoid.
7. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor den organiske syre er valgt fra gruppen bestående af mælkesyre, citronsyre, æblesyre, vinsyre, eddikesyre, ravsyre, isocitronsyre, α -ketoglutarsyre, fumarsyre og oxaloeddikesyre.

8. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor forholdet mellem sukker og organisk syre i drikkevaren er i området 6:1 til 10:1.
- 5 9. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor fremgangsmåden omfatter et yderligere trin f) og/eller et yderligere trin g), hvor trin f) omfatter tilsætning af én eller flere yderligere forbindelser til startvæsken og/eller til AX-REED-væsken og/eller til væsken under fremgangsmåden og/eller til drikkevaren, og trin g) omfatter tilsætning af én eller flere yderligere væsker til AX-REED-væsken, CX-REED-væsken eller REED-væsken.
- 10 10. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor fremgangsmåden yderligere omfatter et trin h) til inkubation af AX-REED-væsken, CX-REED- eller REED-væsken med én eller flere mikroorganismer.
- 15 11. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor i det mindste trin d) og e) gentages mere end én gang.
- 20 12. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor trin d) involverer fjernelse af mindst 30% af mindst én organisk syre.
- 25 13. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor trin e) involverer fjernelse af mindst én kation, indtil der er opnået en ledningsevne på højst 7 mS/cm, fortrinsvis højst 6 mS/cm, endnu mere foretrukket højst 5 mS/cm, fx i området fra 3 til 5 mS/cm.
- 30 14. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor fjernelsen af sure ioner omfatter følgende trin
- I. indsættelse af startvæsken i kammeret til startvæsken; og
 - II. indsættelse af en dialysevæske i de to yderligere kamre til dialysevæsken; og
 - III. påføring af et elektrisk felt over membranstakken;

IV. inkubation af startvæsken i kammeret, hvorved retningen af det elektriske felt skiftes med intervaller, hvorved der opnås en delvist AX-REED-behandlet væske;

V. cirkulering af den delvist AX-REED-behandlede væske til en tank,

5 VI. indsættelse af den delvist AX-REED-behandlede væske i kammeret til startvæsken

VII. påføring af et elektrisk felt over membranstakken;

VIII. inkubation af den delvist AX-REED-behandlede væske i kammeret, hvorved retningen af det elektriske felt skiftes med intervaller,

10 IX. eventuelt gentagelse af trin VI. til VIII.

15. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor fjernelsen af kationer omfatter følgende trin

I. indsættelse af startvæsken, den delvist AX-REED-behandlede væske eller AX-REED-væsken i kammeret til startvæsken eller AX-REED-væsken; og

15 II. indsættelse af en anden dialysevæske i de to yderligere kamre til den anden dialysevæske; og

III. påføring af et elektrisk felt over membranstakken;

20 IV. inkubation af startvæsken, den delvist AX-REED-behandlede væske eller AX-REED-væsken i kammeret, hvorved retningen af det elektriske felt skiftes med intervaller, hvorved der opnås en delvist CX-REED-behandlet væske;

V. cirkulering af den delvist CX-REED-behandlede væske til en tank,

VI. indsættelse af den delvist CX-REED-behandlede væske i kammeret til AX-REED-væsken

VII. påføring af et elektrisk felt over membranstakken;

VIII. inkubation af den delvist CX-REED-behandlede væske i kammeret, hvorved retningen af det elektriske felt skiftes med intervaller,

IX. eventuelt gentagelse af trin VI. til VIII.

DRAWINGS

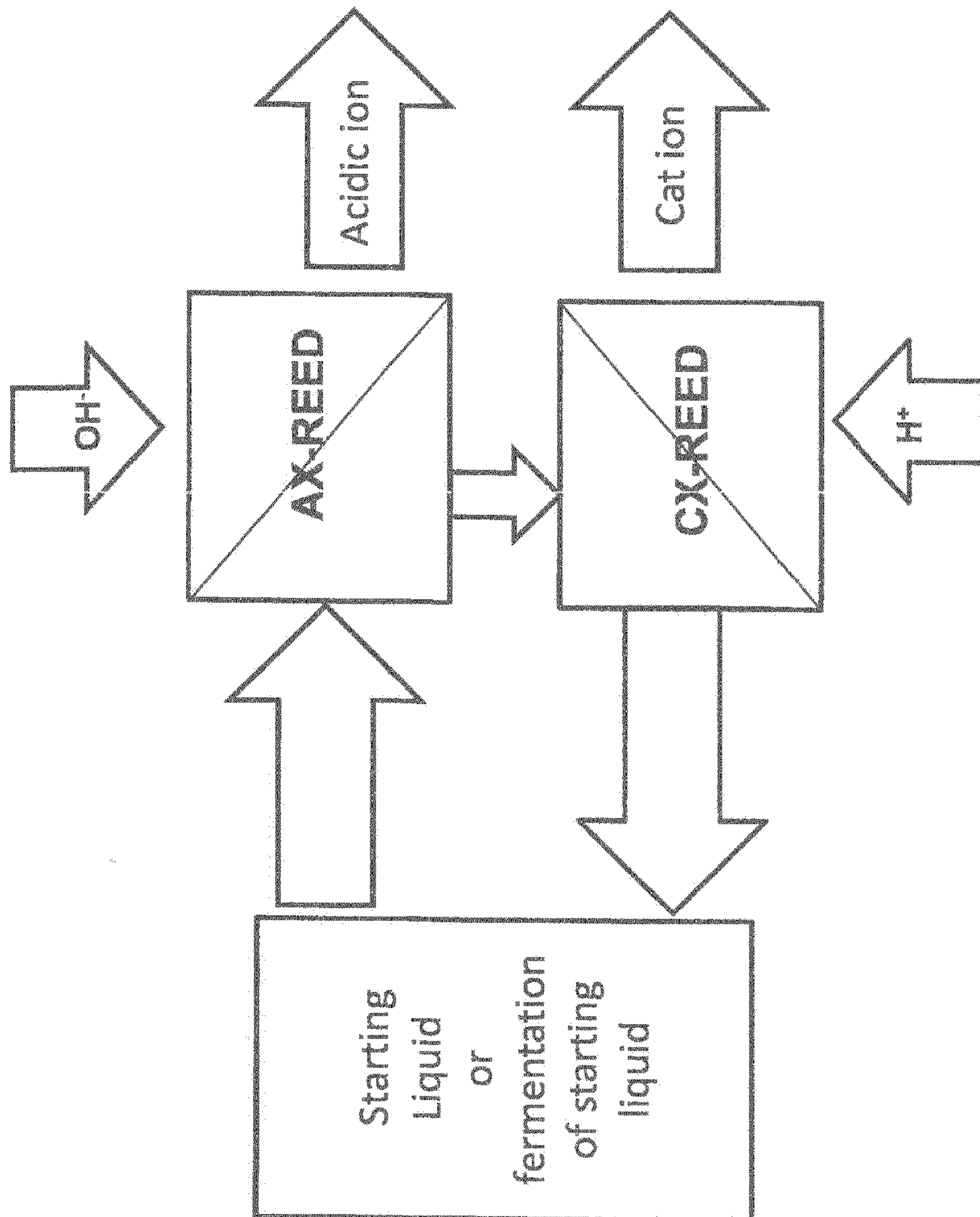
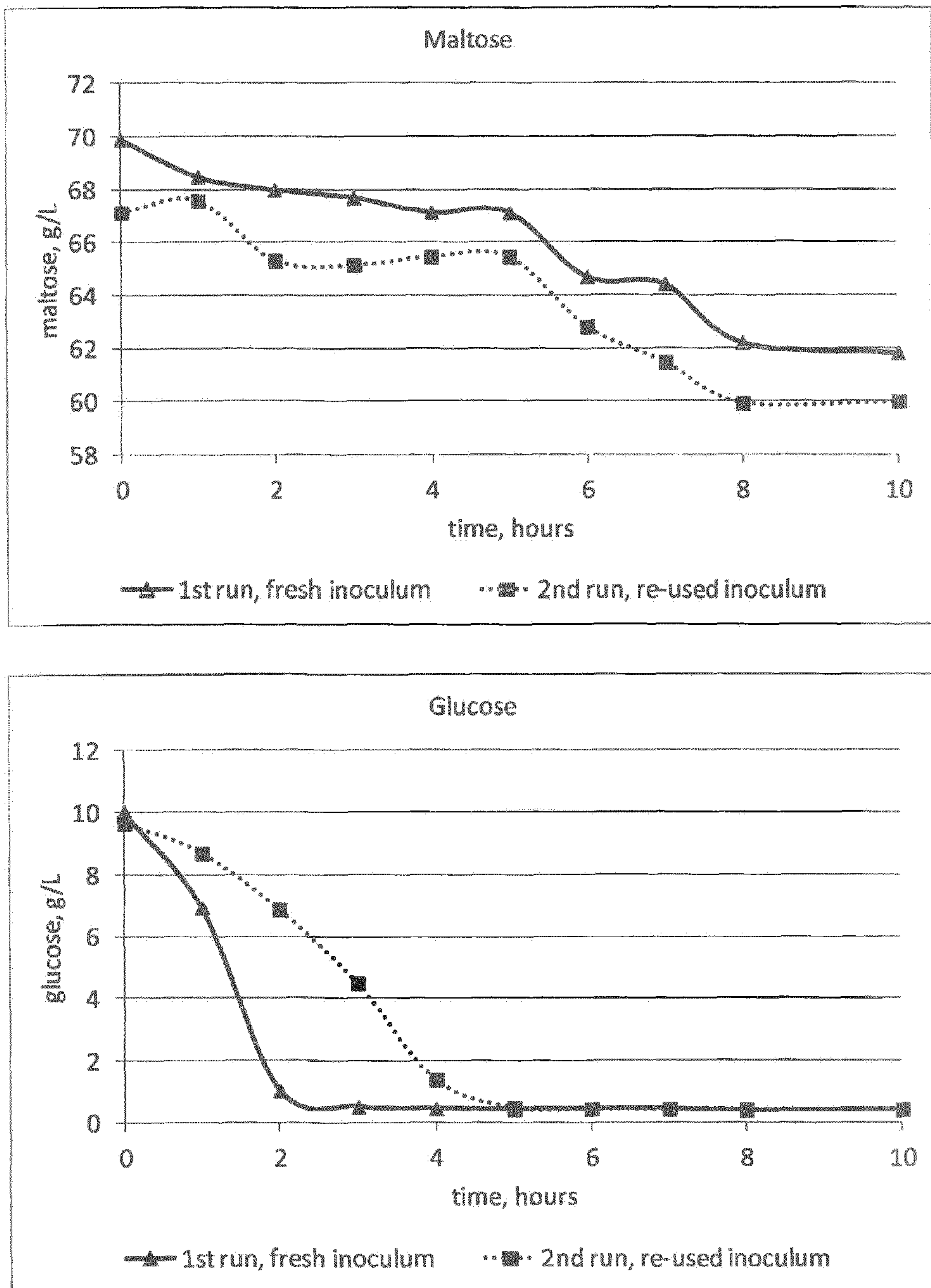
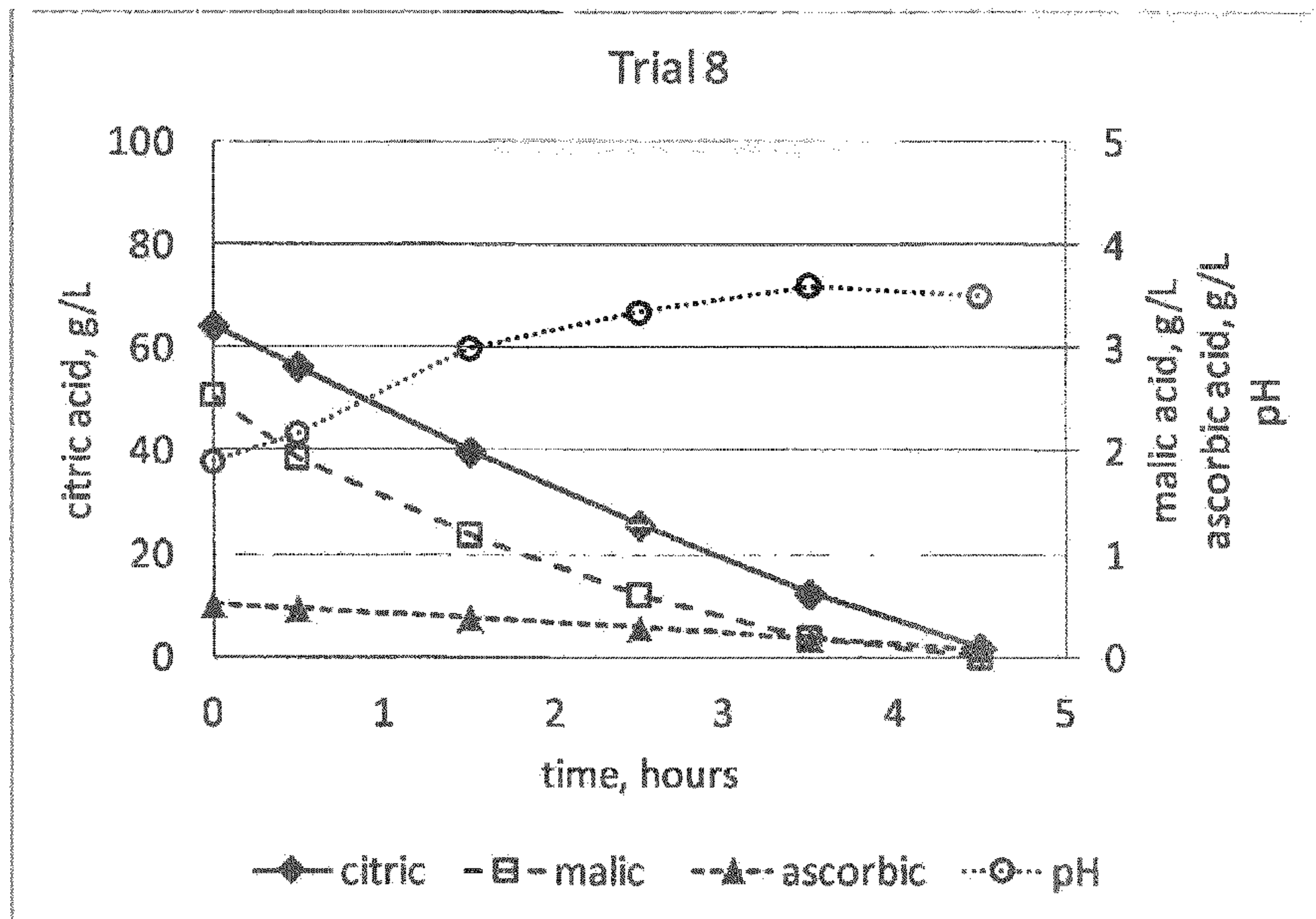


Fig. 1

**Fig. 2**

A)



B)

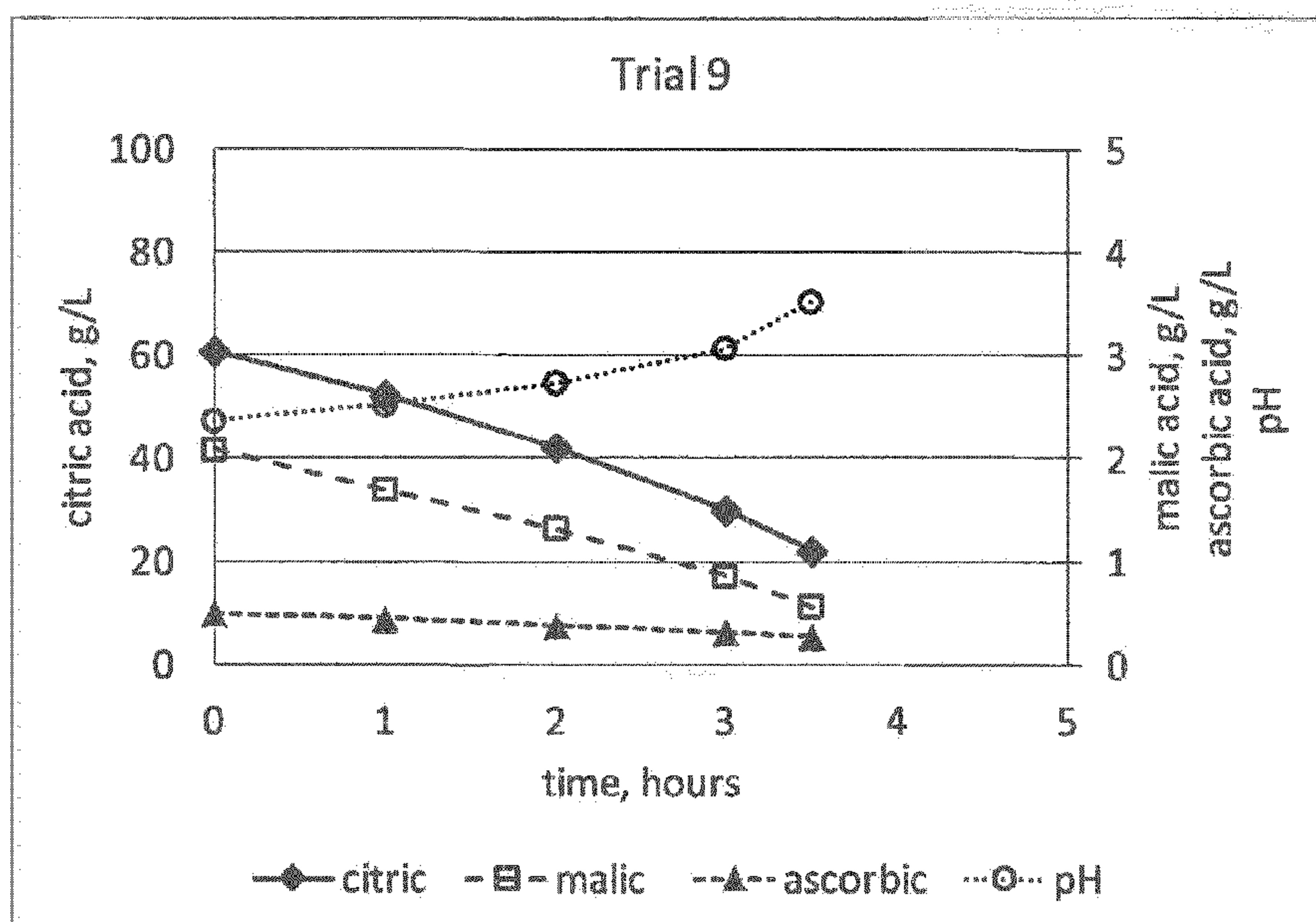
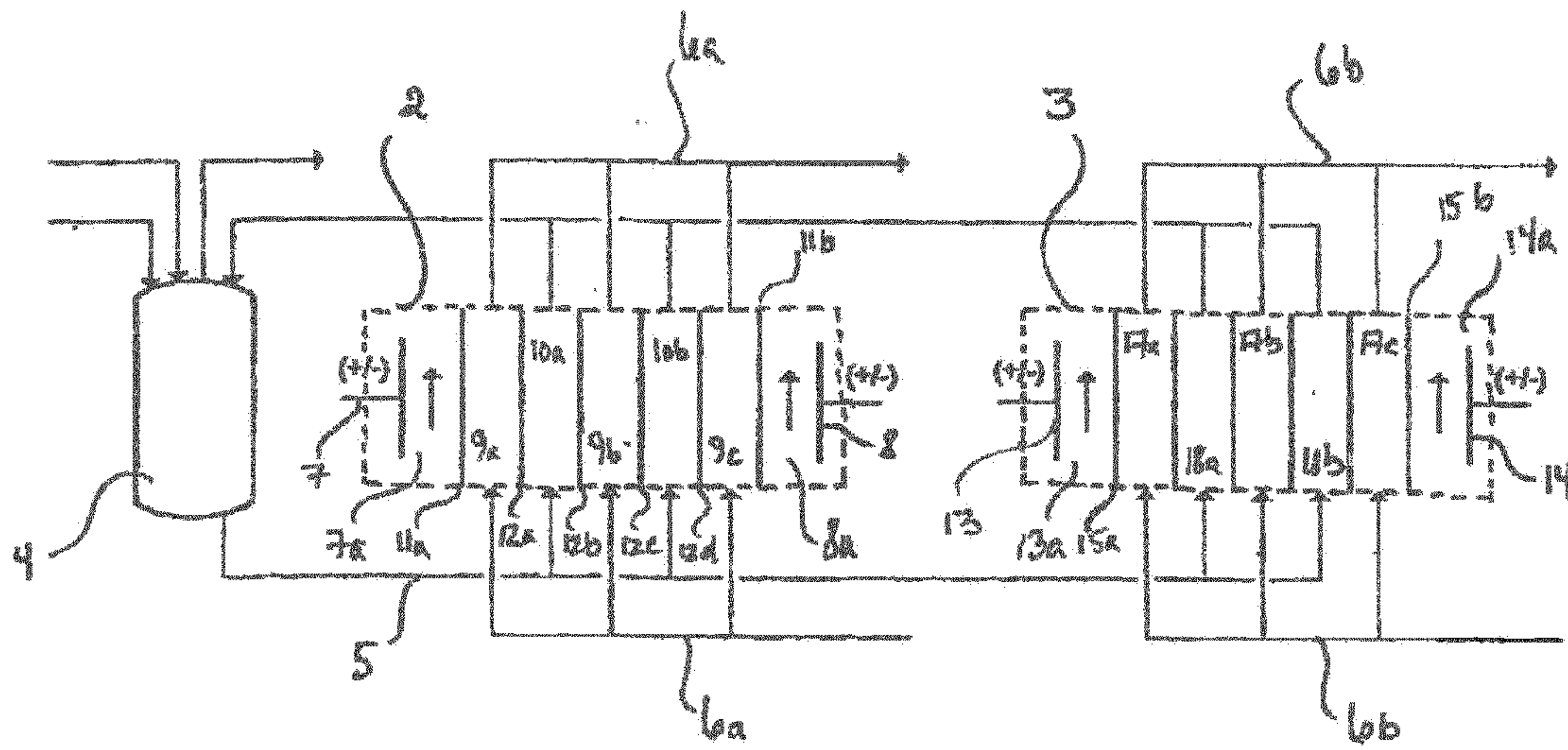
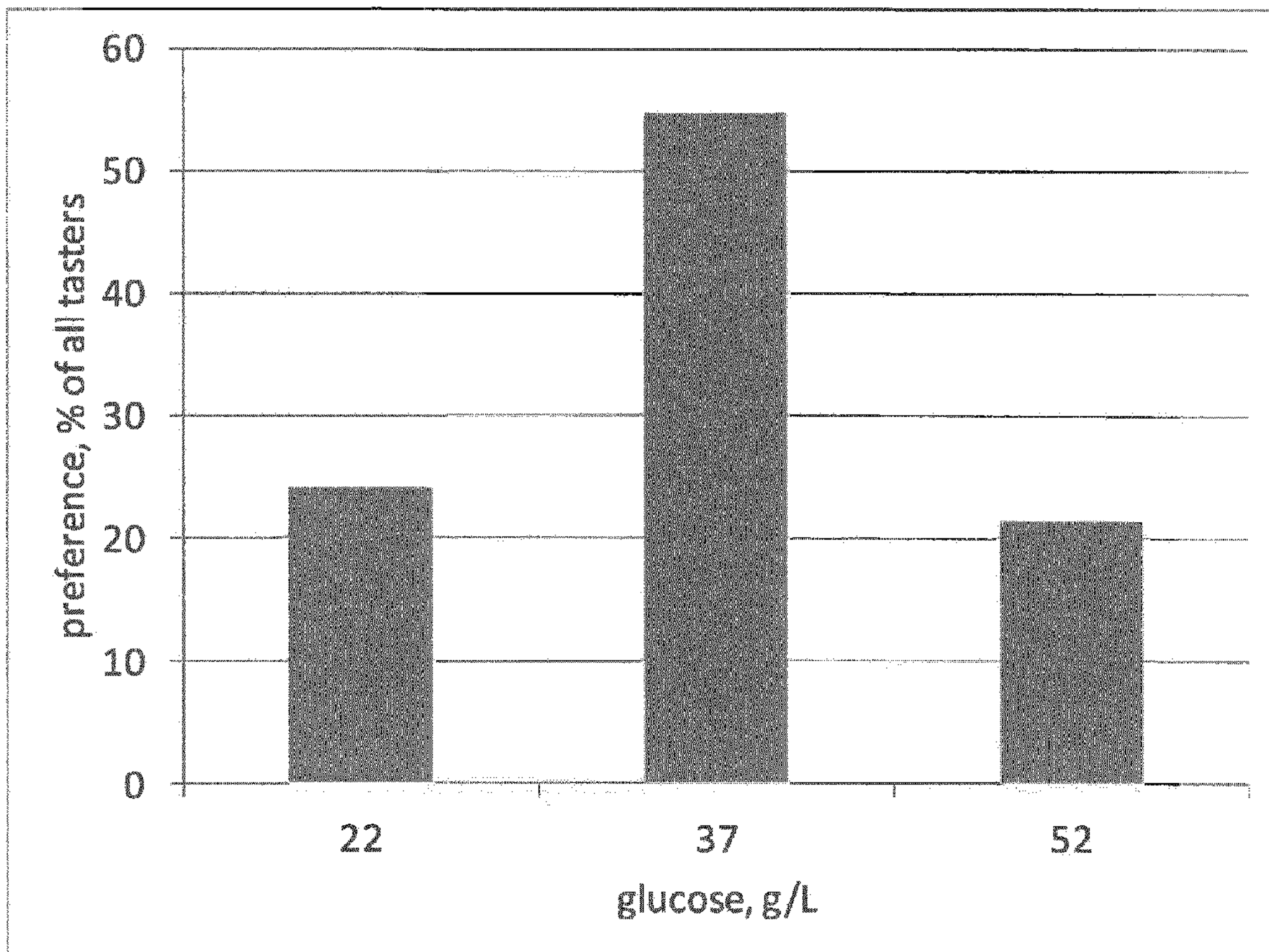


Fig. 3



1

Fig. 4

**Fig. 5**

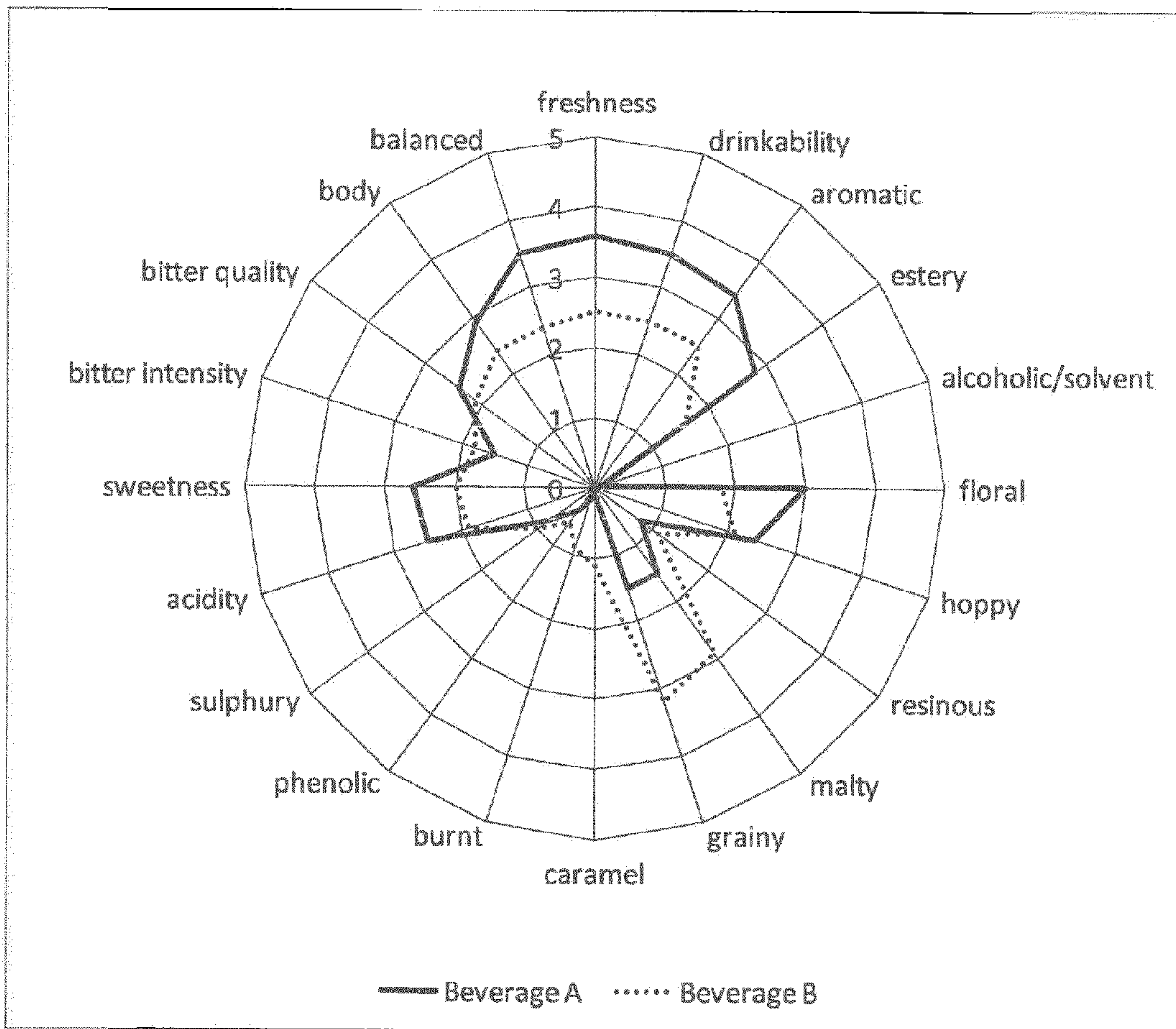
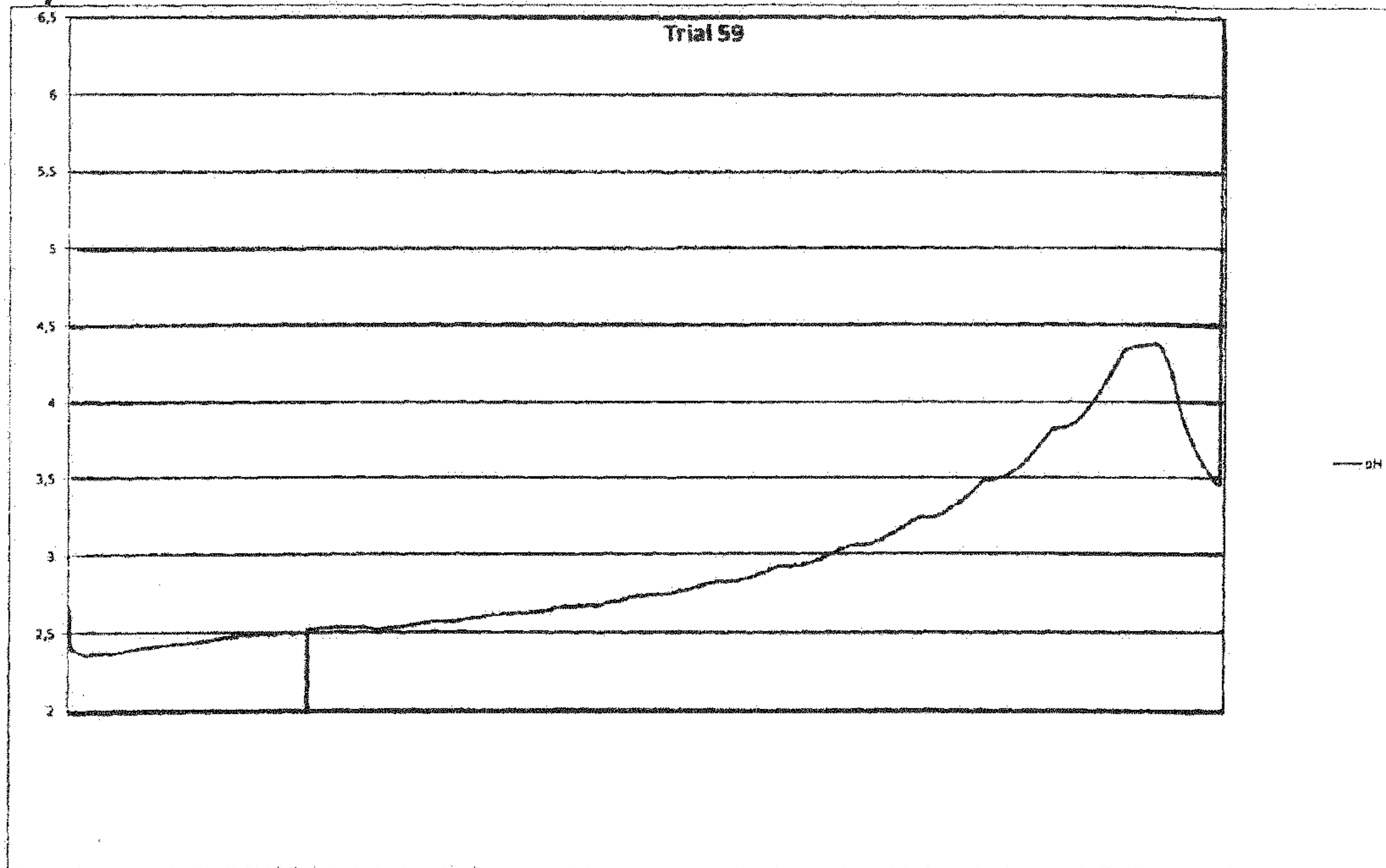


Fig. 6

A)



B)

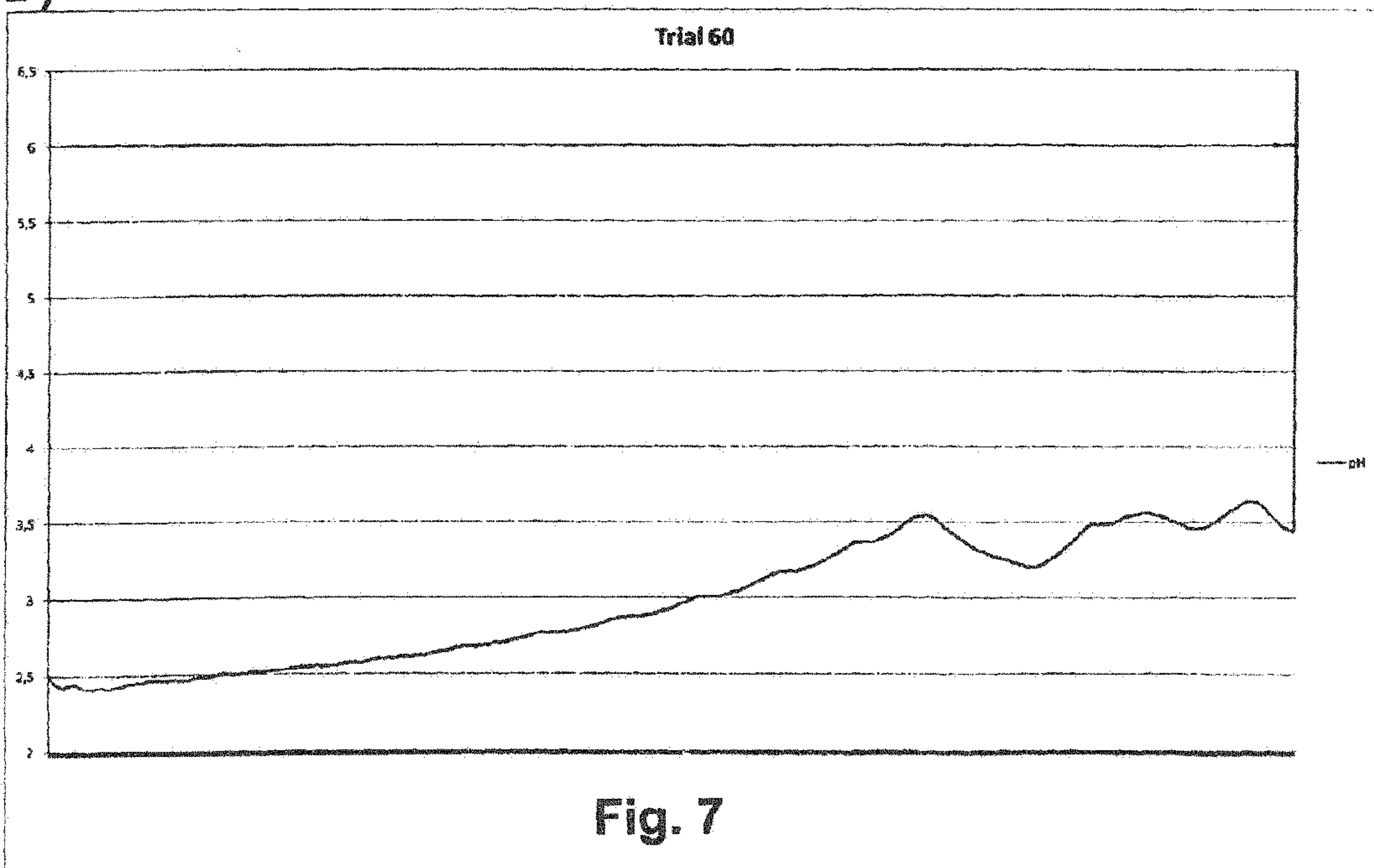


Fig. 7