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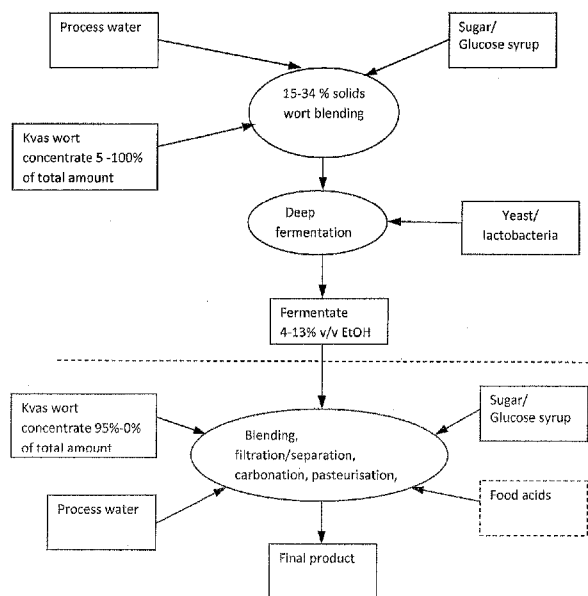
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(54) **Title:** PROCESS FOR THE INDUSTRIAL MANUFACTURE OF KVAS BEVERAGE

Figure 3
New process of kvas production



(57) **Abstract:** The present invention provides a method for the manufacture of a fermented product, which is capable of being mixed with at least sweetener and water to form kvas beverage comprising the steps of either: a) blending kvas wort with sweetener, b) fermenting product of a) with yeast to provide an ethanol content of 2.5%-5% v/v c) separating yeast from product of b), d) distilling fermented separated wort product of c) to produce a concentrated wort with a solids content of at least 65% measured as refractometric Brix and e) heat treating wort concentrate of d); or ai) blending kvas wort concentrate with sweetener and water to produce wort with 15-40%, e.g. 15-34%, solids content, bi) fermenting product of step ai) with yeast to obtain 4-13% v/v ethanol and ci) separating yeast from product of bi). The invention further concerns a method for producing kvas beverage or a syrup which can be diluted to form kvas beverage, together with a concentrated fermented kvas wort, a fermentate, a syrup and kvas beverage obtainable by carrying out the methods of the invention.

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Process for the industrial manufacture of kvas beverage

5 Kvas (or alternatively Kvass) is a fermented beverage which has been a common drink in Eastern Europe since ancient times. It can be produced by the fermentation of extracts from rye malt, rye cereal and/or corn and is popular in countries such as Russia, Ukraine, Belarus, Kazakhstan, Latvia and other Eastern and Central European countries, where many kvas vendors can be found. Kvas is classified as non-alcoholic due to the low level of alcohol present (typically less than 1.2%) and is often flavoured with fruits or herbs.

10 There are several processes known for making kvas, two of which can be used industrially.

The first method is the main industrial method of producing kvas and usually involves two stages; the first stage being the production of kvas wort concentrate and the second stage resulting in the production of the final beverage. The first stage is typically carried out in a specially modified brew house, where raw materials (grist) of fermented rye malt (kilned or freshly germinated) used as a source of flavour and acidity and/or barley malt (which may be used as a source of enzymes), and rye flour or defatted corn flour are used individually or together (blended at any ratio) as carbohydrate sources. The cereals (unmalted materials) are usually processed by pressure cooking to allow fluidisation and starch release and a small amount of barley malt can be added as a source of protease. The cooked cereals are then mashed together with the malts to achieve full saccharification. The malt is then filtered to give kvas wort, concentrated and finally heated (at approximately 110-130°C for 10-30 minutes) to produce the kvas wort concentrate which usually has a solids content of 68-74 refractometric Brix (Plato and Brix are well known units for the measurement of density in the beverage industry). The concentrate can be stored to be used as a raw material for the production of kvas beverage or can be used in other industries e.g. in confectionary or baking.

30 The production of the final beverage takes place in three stages, namely blending, fermentation and separation, and usually occurs within a fermentation plant. The kvas wort concentrate is blended with water (usually about 1-5% w/w) and sugar (usually about 1-7% w/w) (and sometimes with food acids) and is fermented with either only yeast or with acid producing bacteria, such as lactobacteria, and yeast (in which case food acids are not added at the blending

35

stage). The fermentation process should achieve an ethanol content of less than 1.2% v/v (and preferably less than 1% v/v). After fermentation, the microorganisms are filtered from the product which is carbonated and bottled.

5 The second method was recently developed by local beer producers in Eastern European countries to use their available manufacturing capacity after loss of beer market share to multinational companies. They developed a method to produce kvas-like beverages using standard barley malt wort. However, many of the kvas-like beverages produced by local beer producers are reported to taste more like non-alcoholic beer rather than kvas.

10 Thus, currently for the industrial production of kvas, a final beverage fermentation plant is required. Such an operation requires significant investment and highly sophisticated production control. The present inventors have identified a new process for the production of kvas which retains the fermentation process but allows final beverage production in a soft drinks bottling plant i.e. abrogating the
15 requirement for a fermentation plant at the final stages.

The method developed by the inventors involves the production of kvas beverage by mixing concentrated fermented kvas wort with a volatile aromatic fraction captured during the concentration of fermented high gravity wort, sweetener and water (and optionally food acids) or by mixing a fermented kvas wort
20 concentrate (also termed "deep fermentate" herein), preferably comprising 4-13% v/v ethanol, with sweetener, water and optionally unfermented kvas wort concentrate and/or food acids. Thus, the fermentation process is carried out prior to the final mixing of kvas beverage, which allows the final mixing process and final production of kvas beverage to be carried out outside of a fermentation plant.
25 Using the process developed by the inventors, the final beverage can be produced in a standard bottling plant allowing the production of kvas in many different plants, which was not previously possible.

Briefly, the process developed by the inventors allows the production of a fermented product by two different methods where the product can be converted
30 into kvas beverage by a simple mixing step. In a first process, the concentrated fermented kvas wort product may be obtained using kvas wort (the product of filtration of the mash in the industrial kvas process). Kvas wort may be blended with a sweetener and fermented, instead of being concentrated as in the main kvas industrial process used in the prior art. Microorganisms may be separated from the
35 product and the fermented wort distilled. The distillate fraction, preferably

comprising at least 20% v/v ethanol (e.g. 25-60% v/v ethanol) forms the aromatic part of the beverage base and the fermented wort is concentrated preferably to a solids content of at least 65% (measured as refractometric Brix) (e.g. between 68-74% measured as refractometric Brix). The wort concentrate is then heat treated.

5 The wort concentrate and the aromatic parts may be mixed together when it is desired to produce the kvas beverage e.g. at a final bottling plant, and sugar, water (and optionally food acids) may be added. In a second process developed by the inventors, a fermented product preferably with approximately 4-13% v/v ethanol may be obtained by fermenting kvas wort concentrate which may be produced

10 using the conventional prior art kvas industrial method or bought commercially. The kvas wort concentrate to be used in the second process, may be split into two fractions. One fraction may be blended with sweetener and water and fermented deeply by yeast, where the yeast may consume all available sugars and produce ethanol to the limit of their natural ability. The product (deep fermentate) may then

15 be mixed with the other fraction of unfermented kvas wort concentrate, water, sweetener and optionally food acids and may be filtered /centrifuged to remove undissolved solids resulting in the production of kvas beverage. Alternatively, 100% of the kvas wort concentrate may be deeply fermented i.e. the kvas wort concentrate may not be split into two fractions and the final beverage produced by

20 mixing the deep fermentate with water, sweetener and optionally food acids.

The kvas beverage may then be carbonated, pasteurised and bottled. Both of the methods described herein allow the presence of genuine kvas flavour in the final mixed beverage. This is a result of the generation of products of Maillard chemistry occurring during wort heat treatment and the production of volatile

25 aldehydes and esters in yeast fermentation. There is no disclosure in the prior art of a pre-fermented product which may be mixed without further fermentation to produce kvas with genuine kvas flavour. Hence, the present invention allows the fermentation process to be retained but there is no requirement to carry out this process during the final production of the beverage. Particularly, the first method

30 results in the production of a concentrated product that may be easily transported and stored for the production of kvas by a simple mixing step elsewhere.

In a first embodiment, the present invention therefore provides a method for the manufacture of a fermented product, which is capable of being mixed with at least sweetener and water to form kvas beverage comprising the steps of either:

35 a) blending kvas wort with sweetener,

b) fermenting product of a) with yeast to provide an ethanol content of 2.5%-5% v/v,

c) separating yeast from product of b),

d) distilling fermented separated wort product of c) to produce a
5 concentrated wort with a solids content of at least 65% measured as refractometric Brix and

e) heat treating wort concentrate; or

ai) blending kvas wort concentrate with sweetener and water to produce
wort with 15-40%, e.g. 15-34%, solids content measured as refractometric Brix,

10 bi) fermenting product of step ai) with yeast to obtain 4-13% v/v ethanol and
ci) separating yeast from product of bi).

Preferably when steps a) to e) are carried out, the fermented product
(concentrated fermented kvas wort) is capable of being mixed with water, sugar and
optionally food acids and an aromatic solution comprising at least 20% v/v ethanol,
15 e.g. 25-60% v/v ethanol to form kvas and when steps ai) and bi) are carried out, the
fermented product (fermentate comprising 4-13% v/v ethanol) is capable of being
mixed with water and sugar and optionally food acids and/or unfermented kvas wort
concentrate to form kvas.

The term "fermented product" as used herein refers to a product produced
20 from either fermenting a wort with a solids content of 15-40%, e.g. 15-34%,
(produced from kvas wort concentrate) or kvas wort which may be mixed with other
compounds to directly form kvas beverage without the requirement for further
fermentation. The fermented product is not representative of kvas beverage (unlike
in the prior art process where fermentation of blended kvas wort concentrate occurs
25 at the later stages of beverage production i.e. where the fermented product is kvas)
and a further mixing step is required to the fermented product as described in the
present invention to form kvas. As discussed previously, such a fermented product
capable of being mixed with other components to form kvas is not produced in the
prior art methods of kvas production. Thus, the method used in the art in contrast
30 uses kvas wort concentrate in a blending step and then a subsequent step of
fermentation is carried out resulting in the production of kvas. The fermented
product of the art is therefore kvas. The fermented product as prepared in the
present invention may be subsequently mixed to form kvas but the fermented
product itself is not kvas.

35 The water used herein may be "process water".

In one embodiment, the fermented product is a concentrate of fermented kvas wort. This product is the result of (i.e. is obtained or obtainable by) carrying out the method steps a) to e). This concentrated product has a solids content of at least 65 Brix and as discussed above is capable of being mixed with other
5 components (generally water, sweetener, an aromatic solution comprising at least 20% v/v ethanol (preferably a distillate comprising at least 20% v/v ethanol as described below) and optionally food acids to form kvas.

This concentrated fermented product is extremely advantageous since as discussed previously it can be packaged and sent to standard bottling factories for
10 the production of kvas. The pre-fermentation of the product enables kvas to be produced at standard bottling plants without the requirement for fermentation equipment. This is the first concentrated pre-fermented product available for the production of kvas.

In the second embodiment, the fermented product is a fermentate preferably
15 comprising 4-13% e.g. 8-13% v/v ethanol produced by method steps ai) to ci) (referred to herein as the deep fermentate). This product may have variable solids content depending upon quantity of kvas wort concentrate used (may vary from 3 to 20% of real solids) and as discussed above is capable of being mixed with other components (generally water and sweetener and optionally unfermented kvas wort
20 concentrate and/or food acids) to form kvas.

Particularly, in the methods of the invention, the fermented product may be mixed with either an aromatic solution comprising at least 20% v/v ethanol e.g. with 25-60% v/v ethanol or unfermented kvas wort concentrate, together with water, sweetener and optionally food acids to form kvas, as discussed above. Thus, the
25 fermented product produced by method steps a) to e) or ai) to ci) may have different compositions, but both have been pre-fermented i.e. fermented prior to carrying out the mixing step for the final beverage and both are capable of being mixed with other components (at least sweetener and water) to produce kvas without the need for further fermentation.

In a particular embodiment, the invention therefore provides a method for
30 the production of a concentrated fermented kvas wort which is capable of being mixed with sweetener, water, an aromatic solution comprising at least 20% v/v ethanol and optionally food acids to form kvas beverage comprising the steps of a) to e) as described above.

In a further embodiment, the invention provides a method for the production of a fermentate comprising 4-13% v/v ethanol (alternatively viewed as a deep fermentate) which is capable of being mixed with sweetener and water and optionally food acids and/or unfermented kvas wort concentrate to form kvas beverage comprising the steps of ai) and bi) as described above.

The method of producing the fermented product of concentrated fermented kvas wort involving steps a) to e) may also result in the production of an aromatic solution comprising at least 20% v/v ethanol e.g. at least 30, 40, 50, 60, 70, 80 or 90% v/v ethanol. Preferably the aromatic solution comprises 25-60% v/v ethanol e.g. at least 25, 35, 45, 55 or 60% v/v ethanol. Thus, an aromatic solution comprising at least 20% v/v ethanol may be obtained as a fraction during the distillation process (step (d) of the method of the invention). The aromatic solution or fraction may be blended with the concentrated fermented kvas wort to form kvas. An aromatic solution with at least 20% v/v ethanol is preferable for mixing with the concentrated fermented kvas wort in this instance as selecting a fraction with less ethanol results in a fraction with a growing volume which may contain undesirable cereal-type flavours, resulting in an unpleasant aftertaste in the final beverage. The upper limit of ethanol in the solution will vary depending upon the sophistication of the distillation technology.

Thus, in another embodiment this method also provides the production of an aromatic solution comprising at least 20% v/v ethanol e.g. preferably 25-60% v/v ethanol from the distillation step (d). Therefore, as discussed above, the above method steps a) to e) of the invention allow for the production of a concentrated fermented kvas wort and an aromatic distillate comprising at least 20% v/v ethanol which may be mixed to form kvas beverage.

In a further embodiment the invention provides a method for the production of a concentrated fermented kvas wort which is capable of being mixed with sweetener, water, an aromatic solution comprising at least 20% v/v ethanol and optionally food acids to form kvas beverage comprising

- a) blending kvas wort with sweetener,
- b) fermenting product of a) with yeast to provide an ethanol content of 2.5%-5% v/v
- c) separating yeast from product of b),

d) distilling fermented separated wort product of c) to produce a concentrated wort with a solids content of at least 65% measured as refractometric Brix and an aromatic fraction comprising at least 20% v/v ethanol and

e) heat treating wort concentrate.

5 Alternatively viewed, the invention additionally provides a method for the production of an aromatic solution comprising at least 20% v/v ethanol which is capable of being mixed with concentrated fermented kvas wort, sweetener, water and optionally food acids to form kvas beverage, comprising

a) blending kvas wort with sweetener

10 b) fermenting product of a) with yeast to provide an ethanol content of 2.5%-5% v/v

c) separating yeast from product of b)

d) distilling fermented separated wort product of c) to produce an aromatic fraction comprising at least 20% v/v ethanol.

15 As discussed previously, kvas beverage (or syrup) is produced from a final mixing step. The mixing step may preferably include either mixing of the final beverage from all ingredients, preferably followed by carbonation and pasteurisation or a syrup may be prepared from all ingredients which preferably just before bottling may be proportioned with carbonated water to achieve the target
20 density and then pasteurised and bottled or pasteurised in the packaging. The syrup or beverage may need to be filtered or centrifuged to remove any excess of solid particles and this is a standard procedure of the art.

The advantage of this method of production over those reported in the prior art is the ability to blend kvas in a standard bottling plant obviating the requirement
25 for a fermentation plant in the final kvas production steps. The mixing of the concentrated fermented kvas wort and aromatic distillate of the present invention typically involves the addition of sugar or a glucose/fructose syrup and water in a similar way to the prior art blending step. As discussed further below, food acids may also be required for mixing to produce kvas to ensure the beverage acidity is
30 correct. This step may be replaced by an earlier fermentation step using acid producing bacteria, such as lactobacteria, if preferred.

The method steps a) to e) for producing concentrated fermented kvas wort uses "kvas wort" in the initial blending step (a). "Kvas wort" as used herein may be obtained from the initial stages of industrial kvas production known in the art (where
35 kvas wort is generally the filtrate obtained from the initial filtration step in the

standard industrial kvas manufacture process). It is therefore well known in the art how to produce kvas wort. Thus, kvas wort as used in step (a) of the method of the invention may be produced from using a variety of raw materials in the industrial kvas manufacture process. Typically, a source of fermentable carbohydrate is required for kvas wort production, for example, cereals such as rye flour, corn flour e.g. defatted corn flour and/or rice either individually or blended together in any ratio. Alternatively, triticale (a rye/wheat hybrid) may be used as a source of fermentable carbohydrate. The production of kvas wort also requires the addition of enzymes or an enzyme source, to provide amylases (e.g. α -amylase), glucanases, proteases and/or xylanases to the fermentable carbohydrate source. Typically the enzymes used include a thermostable amylase and a beta-glucanase. Protease and xylanase may also be included. Further, pullanase may be included for the destruction of branched amylopectins. Usually, all exogenous enzymes are added to the main mash (suspension of cereals in process water) with the possible exception of protease which could be added to the carbohydrate source during mash-in (preferably at 52°C) to support destruction of the aleuronic layer. A typical enzyme source which may be used is barley malt, although other enzymatic sources may be employed for the kvas wort production.

The cereals or fermentable carbohydrate source to be used are typically treated by pressure cooking under a temperature of approximately 105-110°C (a higher temperature can be used but would provide no efficiency advantage) which allows fluidisation and starch release from the carbohydrate source and a small amount of barley malt may be added to the carbohydrate source as a source of protease (approximately 10% of the total amount (weight) to be used). As discussed above, any other source of protease can be used in place of barley malt.

The cooked carbohydrate (usually cereal) is then typically mashed with fermented rye malt and the remaining barley malt until saccharification is achieved. Saccharification is indicated by negative iodine-starch test. Preferably, full saccharification is to be achieved, however in some cases of difficult raw materials at least 95%, 90%, 80% or 70% of the carbohydrate will be saccharified in the mash stage. Mashing usually occurs between 40-90°C, with the mash-in (addition of cereal to the mash) usually being carried out at approximately 52°C which is optimal for protease activity. Mashing may involve pausing at particular temperatures to allow the activity of particular enzymes. Thus, for example,

pausing at 49-55°C may activate proteases and a pause at 60°C may activate β -glucanase which breaks down β -glucans in the mash. Further, a pause at 65-71°C may allow the conversion of starch to sugar, where a pause at the lower end of the temperature range may favor the production of sugars such as maltotriose, maltose and glucose and a pause near the higher end of the temperature range may favor the production of higher order sugars such as dextrin. Mashing usually takes approximately 2-3 hours. The enzymes used and the pauses at particular temperatures which are used will depend upon the fermentable carbohydrate source which is used for the wort production.

10 Additionally, the pH of the main mash may also be important. For example, if the mash becomes too acidic for enzyme activity, calcium carbonate (or other basic agents like calcium hydroxide or potassium bicarbonate etc) may be added to allow the pH to increase above 5.

15 The mash is then subjected to filtration with mash filters to remove spent grist, where the filtrate obtained is kvas wort. Two filtration systems are generally used in this respect, namely Meura or Ziemann. Both of the filtration systems are suitable for kvas wort filtration, although Meura may be preferable.

20 Kvas wort is usually a mixture of heavy wort and sparge water. The heavy wort drawn off the mash filter may have solids up to 23 Brix, and this is usually then topped with hot sparge water used for recovery soluble solids from filter cake. Thus final solids content in ready wort is typically an average of 16-18 Brix, though in extreme cases of massive sparging it may be as low as 9 Brix.

25 Thus, although kvas wort may be obtained by any appropriate process, it can be produced as described above for use in the present invention. An alternative method for kvas production is described in WO 2011/026591 which is incorporated by reference herein. Kvas wort is typically produced for immediate use in step a) of the method of the present invention as it is a microbiologically sensitive product. However, kvas wort may also be stored before use in step a) by freezing or kvas wort may be sterilised and aseptically packaged.

30 The solids content of the kvas wort used in step a) may be variable e.g. it is possible to use kvas wort with a solids content of 16-18 Brix or as low as 9 Brix as indicated above. The amount of kvas wort to be used will depend on the volume of beverage being produced, the final desired solids content of the beverage and the solids content of the kvas wort. Thus, the amount of kvas wort to be used can easily
35 be calculated once the volume and kvas wort solids content of the beverage have

been decided, and the solids content of the starting kvas wort is known. As discussed below, a wort solids content of 1-5% may be typically present in the beverage e.g. 1-4, 1-3, 2-4, 2-3 or 2%.

5 The term "wort" as used herein refers to either cereal extract obtained by filtration of mash diluted or undiluted with sparge water, or diluted to single strength concentrated extract with or without addition of other beverage ingredients specified herein. Sparge water is a diluted extract generated by collecting water that has been filtered through the drained mash to extract additional sugars. The term "wort" is used herein interchangeably to mean kvas wort or kvas wort concentrate or a
10 product obtained from treating kvas wort or kvas wort concentrate e.g. after a blending, mixing, heating, fermentation, distillation step or a mixture of these.

The term "sweetener" as used herein refers to any sugar (sweet saccharide) source and for example encompasses sucrose and syrups of glucose and/or fructose as well as oligo- and polysaccharides comprising monosaccharide
15 monomers. When the sweetener is blended in either step a) or ai) of the methods discussed above, the main requirement is for the sweetener to provide nutrition for the yeast for fermentation. For step ai) the amount of sweetener should be sufficient to allow the production of from 4%-13% v/v ethanol in the fermentate. In order to achieve that, preferably 8-26% of solids should be sweeteners such as
20 glucose. For step a), the target ethanol content after fermentation is lower, 2.5-5% v/v (preferably 3-4% v/v) and thus approximately 3.5-6.5% glucose equivalent sweetener solids (preferably 4-6% glucose equivalent sweetener solids) may be added.

The fermented products are also capable of being mixed with sweetener to
25 produce kvas, as discussed previously. In the mixing step, sweetener may be added to the product of step e) or bi) as discussed in detail below.

The term "kvas wort concentrate" or "concentrated kvas wort" as used herein refers to any concentrated form of kvas wort, as defined above. Concentration may be carried out by any process known in the art and typically is
30 carried out using a two or three effect evaporation plant. In step d) of the method of the invention, the first effect is combined with using the distillation column which separates the most volatile fraction with an ethanol content of at least 20% v/v. Typically, kvas wort concentrate may be obtained by concentrating kvas wort or a fermented form of kvas wort to a solids content of at least 65 expressed as
35 refractometric Brix. Such a solids content should ensure a low water activity and

high acidity which may prevent microbiological contamination. Usually kvas wort concentrate may have a solids content of from 68-74 refractometric Brix e.g. at least 68 refractometric Brix.

5 The term "deep fermentate" as used herein refers to the product of method steps ai) and bi) of the invention which typically is a product of deeply fermenting wort and which may comprise 4-13% v/v ethanol e.g. 5, 6, 7, 8, 9, 10, 11, 12 or 13% v/v ethanol. "Deep fermentation" is a fermentation process carried out with yeast wherein the yeast are allowed to utilise all available sugars (e.g. at least more than 90% of sugars).

10 The sweetener as used in step (a) of the method of invention is blended with kvas wort. Typically the sweetener is easily dissolved in kvas wort. The sweetener can also be used in a further step f) which is discussed further below.

The amount of sweetener to be added to step a) can be calculated based on the total volume of kvas beverage being produced in the method together with the target ethanol content. A model calculation is shown in Example 1. Thus, briefly the mass and solids content of the kvas wort being used in the method can be used to calculate the final volume of kvas beverage which may be produced (e.g. based on a beverage comprising 1-5% wort solids). Alternatively viewed, the volume of beverage to be produced can be decided together with the final % wort solids in the beverage and the required amount of kvas wort with a particular solids content which is needed to make that beverage can be calculated. This volume of beverage together with the target ethanol concentration in the beverage e.g. 0.5% v/v can then be used to calculate the total amount of ethanol that should be produced in the method. As it is known that 342g of sucrose is converted into 184g of ethanol in a biochemical reaction, the amount of sweetener which needs to be added to produce the total amount of ethanol needed can easily be calculated.

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As discussed in step (b) of the method of the invention, the blended kvas wort of step (a) is then subjected to a fermentation step with yeast in order to reach an ethanol content of between 2.5-5% v/v and preferably between 3-4% v/v. Alternatively viewed, the ethanol content may be at least 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9 or 5.0 % v/v. The yeast which can be used in this fermentation step are typically *Saccharomyces cerevisiae* (baker's and brewing cultivars) but other yeasts may also be used. For example, suitable yeasts may be selected from the list comprising

30
35 *Saccharomyces uvarum*, *Saccharomyces bayanus*, *Saccharomyces pastorianus*

(also known as *Saccharomyces carlsbergensis*), *Schizosaccharomyces pombe*, *Xanthophyllomyces dendrorhous*, *Debaryomyces hansenii*, *Hanseniaspora uvarum*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Pichia angusta*, *Pichia anomala*, *Brettanomyces* and *Torulaspora delbrueckii*. Although preferably a pure yeast
5 culture is used in the fermentation i.e. a yeast culture comprising only one yeast species or one yeast cultivar, a mixture of different yeast may also be employed e.g. 50% *S.cerevisiae* baker's cultivar or brewer's cultivar and 50% *S.uvarum*. In a preferred embodiment, *S.cerevisiae* is used as the yeast culture in the methods of the invention. *S.cerevisiae* (or any other yeast) may be used in both dry and
10 suspended forms. The pitching (inoculation) rate of yeast used in method step b) is preferably about 10M cell/g of preparation and in step bi) is preferably about 75M cell/g of preparation.

The fermentation step b) may be carried out using the following conditions. As discussed above, dry yeast may be used in fermentation step b), where dry
15 yeast may be rehydrated before inoculation. For rehydration preferably 10% w/w suspension of dry yeast may be prepared at a temperature of about 30°C. Rehydration can be carried out in water solution of fermentable carbohydrates; or in diluted or undiluted wort; or just in process water. Such a suspension may be gently mixed to ensure that all yeast is in rehydrated form. The yeast suspension is
20 typically left for 30 minutes (e.g. at least 10, 20 or 30 minutes) and the hydrated yeast may then be added to the product of step a). Fermentation process step b) may be carried out in cylindrical-conical vessels typically used in brewing which allow aeration. Fermentation step b) may be particularly carried out at 25-30°C and preferably at 28°C. Fermentation process needs to be supported by appropriate
25 aeration. Fermentation step b) may be stopped artificially by chilling and separating yeast when target ethanol content is achieved e.g. 2.5-5 % v/v. In fermentation step bi) (discussed in more detail further below), the yeast may decline naturally when the concentration of ethanol exceeds 8% v/v. Fermentation may take from 8 to 48 hours, depending upon specific conditions.

30 As discussed above, the fermentation is carried out until a particular concentration of ethanol is achieved in the product. The ethanol % may be measured by a combination of densitometry and infrared spectroscopy and particularly, the % ethanol may be measured using Alcolyzer Beer Plus and density meter DMA 4500 (Anton Paar, Austria).

An additional fermentation step, a so-called "bacterial" fermentation step, may also be carried out in the present methods using acid producing bacteria. "Acid producing bacteria" as used herein refers to bacteria capable of producing organic acids, particularly the food acids as defined elsewhere herein. Thus, acid producing bacteria are selected from acetic acid bacteria, propionic acid bacteria, *Bifidobacteria* and lactic acid bacteria (also known as lactobacteria). In a preferred embodiment, the additional "bacterial" fermentation step is a lactobacterial fermentation step, i.e. using lactic acid bacteria. Such an additional bacterial, preferably lactobacterial, fermentation step may be carried out either prior to, at the same time as or after the yeast fermentation. Bacterial fermentation carried out at the same time as the yeast fermentation may encompass either a combined fermentation of the product of step a) (or step ai)) with both yeast and acid producing bacteria, preferably lactobacteria, or may involve splitting the product of step a) (or step ai)) and allowing yeast fermentation on one part and bacterial fermentation on the other part. This later fermentation may also be referred to as a parallel fermentation i.e. where separate fermentations are carried out using yeast and bacteria, preferably lactobacteria, on a split product from step a). Thus, in this later embodiment, the yeast and bacterial fermentations are carried out separately but possibly at the same time. (Alternatively, such a split or parallel fermentation may occur at different times). Once the fermentation reactions have reached their target ethanol % or acidity, the split fermentation products may be united or mixed again.

However, in a preferred embodiment for the method comprising steps a) to e), the bacterial, preferably lactobacterial, fermentation step may be carried out prior to fermentation with yeast.

Thus, the method(s) of the invention may include two fermentation steps, where one fermentation may be carried out with acid producing bacteria, preferably lactobacteria, and the other fermentation may be carried out with yeast. The bacterial fermentation may be carried out to produce food acids in the wort and particularly, an acidity of 0.2-0.7% w/w expressed as citric acid is preferred, particularly 0.2, 0.3, 0.4, 0.5, 0.6 or 0.7% w/w. Alternatively, if the bacterial fermentation step is not carried out, it is possible to add food acids at the final mixing stage of the kvas beverage. Thus, the fermentation with acid producing bacteria, preferably lactobacteria, is an optional step in the production of the concentrated fermented kvas wort and/or distillate/aromatic solution comprising

ethanol (method steps a) to e)) (and is also optional in method steps ai) and bi)) which can be obviated by including food acids at the final mixing stage to form kvas.

The acid producing bacteria which can be used in the optional fermentation step may be lactobacteria, *Bifidobacteria*, propionic acid bacteria or acetic acid bacteria. The lactobacteria may be selected from the list comprising *Lactobacillus acidophilus*, *Lactobacillus amyolyticus*, *Lactobacillus amylovorus*, *Lactobacillus alimentarius*, *Lactobacillus aviaries*, *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus casei*, *Lactobacillus crispatus*, *Lactobacillus curvatus*, *Lactobacillus delbrueckii*, *Lactobacillus farciminis*, *Lactobacillus fermentum*, *Lactobacillus gallinarum*, *Lactobacillus gasseri*, *Lactobacillus helveticus*, *Lactobacillus hilgardii*, *Lactobacillus johnsonii*, *Lactobacillus kefiranofaciens*, *Lactobacillus kefiri*, *Lactobacillus mucosae*, *Lactobacillus panis*, *Lactobacillus paracasei*, *Lactobacillus paraplantarum*, *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Lactobacillus pontis*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus sakei*, *Lactobacillus salivarius*, *Lactobacillus sanfranciscensis*, *Lactobacillus zaeae*, *Lactococcus lactis*, *Leuconostoc citreum*, *Leuconostoc lactis*, *Leuconostoc mesenteroides*, *Pediococcus acidilactici*, *Pediococcus dextrinicus*, *Pediococcus pentosaceus* and *Streptococcus thermophiles*. In a particularly preferred embodiment the lactobacteria may be *Lactobacillus paracasei*, *Lactobacillus casei* or *Lactobacillus brevis*. The acetic acid bacteria may be selected from the list comprising *Gluconoacetobacter hansenii*, *Gluconoacetobacter intermedius*, *Gluconoacetobacter liquefaciens*, *Gluconoacetobacter xylinus*, *Gluconobacter cerinus*, *Gluconobacter oxydans*, *Acetobacter cerevisiae*, *Acetobacter pasteurianus* and *Acetobacter oeni*. An example of a propionic acid bacteria suitable for use in the methods of the invention is *Propionibacterium freudenreichii*. *Bifidobacteria* may be selected from the list comprising *Bifidobacterium adolescentis*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium breve* and *Bifidobacterium longum*.

The fermentation may be carried out using the vessels, temperatures and times discussed above with respect to the yeast fermentation step and as discussed above, an acidity of 0.2-0.7% w/w is preferably obtained from the fermentation. Acidity may be measured by titration of 100g or 100ml of beverage sample with 0.1N solution of NaOH until pH 8.11 is achieved. The volume of NaOH used is automatically recalculated into concentration of citric acid present and this method is commonly used to determine acidity for many beverages produced in the

industry e.g. by Coca-Cola. Acidity is often expressed as ml of 0.1N or 1N NaOH required for titration until pH 8.11. Step (c) of the method of the invention requires the separation of yeast after the fermentation process has been completed.

5 Separation may be carried out by filtration but preferably separation is by centrifugation after the fermentation step or steps. Any other means of separating yeast can also be employed e.g. decanting the liquid product of the fermentation process from the precipitated yeast after chilling to about 4°C. A combination of separation techniques may also be used in step c), particularly, a step of centrifugation may be followed by filtration e.g. membrane filtration, in order to
10 remove any residual microorganisms left over from the first separation step. Alternatively, separation e.g. by centrifugation, may be followed by pasteurisation to ensure the elimination of residual microorganisms.

As discussed above, centrifugation may be preferably employed to separate yeast from the fermentation product of step (b). Such centrifugation can typically be
15 carried out using industrial sized centrifuges to process approximately 20000-30000 l/hour. Typical separation equipment is supplied by GEA Westfalia or Alfa Laval. Preferably, the product of step b) is chilled before any step of centrifugation is carried out. Particularly, the product of step b) may be chilled to less than 10°C where such conditions may inactivate yeast.

20 Several different filters or membranes may be used if filtration is employed to separate yeast from the fermentation product, including pre-made filtration media such as sheets. A fine powder (for example diatomaceous earth) may also be used which can be introduced into the fermentation product and recirculated past screens to form a filtration bed. The filters/membranes may be rough, or fine, but,
25 preferably the filters/membranes are sufficiently fine to enable at least 5 –log reduction of the yeast from the fermentation product. The selection of a suitable filter is well within the competency of someone skilled in this field. A typical membrane/filter pore size may be 0.45 microns. It will be appreciated that filters are manufactured and marketed as having a particular pore size but the
30 manufacturing process may occasionally result in a few smaller or larger pores. Thus the pore size is a reference to the diameter of the most common pore size of a filter. Sterile filters can also be used which allow almost all microorganisms to be removed from the fermentation product. In such a case, at least 5-log reduction of yeast may be achieved in the fermentation product.

Step (d) of the method of the invention requires the distillation of the fermented separated wort product of step (c) which may be the filtrate if filtration was employed as a means of separating yeast or the supernatant if a step of centrifugation was employed as a means of separating yeast. A distillation column with plates or with packing may be used in step d). It is possible to obtain a higher concentration of ethanol in the distillate by increasing the number of plates in the column. The distillation process allows the concentration of fermented kvas wort, particularly to a solids content of at least 65 % w/w measured as refractometric Brix and preferably to a solids content of 68-74% w/w measured as refractometric Brix and also allows the separation of a liquid fraction comprising at least 20% v/v ethanol as previously discussed. This fraction is referred to herein as the distillate or as an aromatic solution comprising ethanol. Thus the aromatic solution referred to herein contains ethanol as described hereinbefore and one or more aromatic compounds, i.e. having aroma, e.g. as described in the table below. Typical aromatic components in the distillate of the invention are as follows:

Compound	Distillate
Diacetyl (ppm)	3.5
Pentanedione (ppm)	2.7
Total VDK (ppm)	6.2
Ethyl acetate (ppm)	21.7
Isoamyl acetate (ppm)	1.1
Total esters (ppm)	22.8
Propanol (ppm)	67.9
Isobutanol (ppm)	155.9
Isoamyl alcohol (ppm)	609.8
Total fusel alcohol (ppm)	833.6
Acetaldehyde (ppm)	54.4

However, it will be appreciated that the amounts and types of aromatic components present will vary and the aromatic solution of the invention may contain any one or more of the listed aromatic compounds.

The method of the invention also requires step (e) i.e. heat treatment of the concentrated wort obtained in step (d). The heat treatment can be carried out using any heat source although in practice direct steam and/or indirect heating with a jacket or coil with heating media may be used. Typically a temperature between
5 110-130°C may be employed for 10-30 minutes. In the case of indirect heating with a jacketed heat exchanger, this may be equipped with rolling blades scrapping the internal surface to prevent local burning of concentrated wort and consequent surface faulting. This heating process enables the concentrated wort to reach the target colour and flavour profile so that it is able to be blended as discussed further
10 below to form kvas beverage.

Thus, the first method of the invention results in the production of a concentrated fermented kvas wort and an aromatic solution (or distillate) comprising at least 20% v/v ethanol which may be mixed to form kvas beverage. Hence, a further step of mixing the concentrated fermented kvas wort and aromatic solution
15 comprising at least 20% v/v ethanol, together with water (which may be obtained from earlier process steps), sweetener and optionally food acids may be carried out. In a further embodiment, the invention therefore provides a method for the production of kvas beverage comprising the steps of:

- a) blending kvas wort with sweetener,
- 20 b) fermenting product of a) with yeast to provide an ethanol content of 2.5-5% v/v,
- c) separating yeast from product of b),
- d) distilling fermented separated wort product of c) to produce an aromatic solution comprising at least 20% v/v ethanol and a concentrated wort with a solids content of at least 65% measured as refractometric Brix,
- 25 e) heat treating concentrated wort and
- f) mixing product of e) with water, sweetener and optionally food acids and with the aromatic solution comprising at least 20% v/v ethanol of d).

The mixing step f) involves the mixing of the heat treated concentrated wort (or alternatively viewed the concentrated fermented kvas wort), water and
30 sweetener e.g. sugar or glucose and/or fructose syrup as detailed above. The mixing step may be undertaken in any suitable tank with an impeller or circulation pump where ingredients may be dosed manually or with the aid of any type of automation. Typically the sweetener (e.g. sugar or glucose syrup) may be added so that the final product contains from 1 to 3% solids from sugar or equivalent
35 sweetener. Thus, preferably the final product may contain from 4 to 8% solids of

which 1-3% is from sugar and 1-5% is from concentrated fermented kvas wort. The rest of the final product (preferably from 92-96%) may be water where the water may or may not be obtained from earlier processing steps.

As discussed previously, food acids are only included in the final mixing step if an earlier fermentation with bacteria, preferably lactobacteria, was not carried out. Food acids may also be included if only a partial bacterial fermentation was carried out e.g. if insufficient acids were produced by the fermentation. Food acids which may be added in mixing step f) are edible organic acids and particularly include lactic acid. Citric, acetic, tartaric and malic acids may also be used. One or more of the food acids may be used in the final mixing step e.g. a combination of acids can be used if desired. Such food acids are commercially available. The food acids are added to reach an acidity of between 0.05-0.25% w/v (particularly 0.05-0.25 %w/v expressed as citric acid), particularly between 0.1-0.2% w/v. As previously discussed, the food acids may not be included in the mixing step f) if a further fermentation step is included in the method using acid producing bacteria, preferably lactobacteria.

The aromatic solution is also mixed in step f), preferably after the initial mixing of the heat treated concentrated wort, water, sugar and optional food acids. The aromatic solution is added to reach an ethanol content which is preferably within the range of 0.4-1%, but may be up to 1.2%.

Kvas final beverage may however be produced by 2 conventional methods of beverage industry. Firstly, the beverage can be mixed directly to single strength (4.5 -10 apparent Brix e.g. 5-10, 6-10, 7-10 ,8-10, 5-9, 6-9, 7-9, 5-8, 6-8) as described above. Alternatively, it can be made through an intermediate final syrup which includes all ingredients but only a fraction of water (syrup water). Thus, the fraction of water used to produce the syrup is termed "syrup water". This final syrup may contain 15-50% of solids e.g. 20-40, 20-35, 20-30, 30-40, 35-40% and thus alternatively viewed is a more concentrated form of kvas. At bottling, the final syrup may be mixed with still or carbonated water (beverage water) using special equipment referred to as proportioner. The mixing ratio may vary from 2 to 6 volume of water per 1 part of syrup e.g. 3, 4, 5 volumes of water. The invention therefore further provides a method of making a syrup which can be diluted with still or carbonated water to form kvas, comprising at least steps a) to e) above, and preferably steps a) to f) to produce a syrup with 15-50% solids content. Syrup or

beverage may be additionally treated by bubbling of sterile air to reduce undesirable off-notes.

Therefore, the invention also provides a method for producing a syrup which can be diluted with still or carbonated water to form kvas comprising the steps of

- 5 a) blending kvas wort with sweetener,
- b) fermenting product of a) with yeast to provide an ethanol content of 2.5%-5% v/v
- c) separating yeast from product of b),
- d) distilling fermented separated wort product of c) to produce a
10 concentrated wort with a solids content of at least 65% measured as refractometric Brix,
- e) heat treating wort concentrate of d) and
- f) mixing product of e) with sweetener, water and optionally food acids and an aromatic solution comprising at least 20% v/v ethanol to form a syrup comprising
15 a solids content of 15-50% measured as refractometric Brix.

As indicated above, the water used in step f) for producing the syrup is known as "syrup water".

Preferably, in step f), beverage quality water is used for the mixing.

"Total water" refers to the whole amount of water used for mixing of final
20 beverage. The fraction of total water used for mixing of a syrup intended for further dilution is referred to as "syrup water". The fraction of total water used for dilution of syrup to final beverage is referred to as "beverage water". Thus, the total water in the final beverage may comprise syrup water plus beverage water. If final beverage is mixed directly from ingredients, i.e. not via the syrup intermediate, the
25 total amount of water may be used in that mixing step.

"Beverage quality water" refers to water which meets all safety requirements and in which alkalinity is preferably reduced below 85 ppm expressed as CaCO_3 . Beverage quality water may therefore have been purified e.g. by filtration or UV radiation, to remove impurities such as unwanted minerals or organic materials.
30 Beverage quality water has preferably been subjected to steps such as enhanced filtration to remove pathogenic organisms, disinfection (provides a secondary barrier against microorganisms), carbon purification and/or polishing filtration. Preferably, the total water, i.e. the beverage water and syrup water is of beverage quality.

Further steps may be included within the method for the production of kvas beverage, including steps of carbonating the beverage and/or pasteurising the beverage. The beverage may also be bottled or alternatively packaged into kegs (beer draft containers). Steps of preparing the kvas wort used in step a) may also
5 be employed in the first method of the invention. Such steps are discussed above in relation to "kvas wort".

Thus, as discussed previously, the first method of producing kvas of the present invention allows production of a heat treated concentrated wort and an aromatic solution which can be mixed to form the final beverage (or a syrup) without
10 the need for fermentation equipment.

The second production method of the invention requires the performance of steps ai) to ci) as discussed above, where step ai) requires the blending of kvas wort concentrate with sweetener and water to produce a wort with 15-40%, e.g. 15-34%, solids content (preferably 17-30% solids content), bi) requires fermenting the
15 product of step ai) with yeast to preferably obtain 4-13% v/v ethanol and ci) requires separation of yeast from the product of bi).

Preferably in this second method, the kvas wort concentrate used in step ai) may be obtained using the initial stages of the prior art industrial method for kvas production e.g. by the concentration of kvas wort to a solids content of at least 65 %
20 measured as refractometric Brix and by the heating of said product. The heating step may be carried out at 110-130°C for 10-30 minutes to produce kvas wort concentrate. Kvas wort concentrate as used in step ai) is also commercially available, for example from CJSC "ATRUS", Rostov town, Yaroslavl Region, Russia or from CJSC "Kostroma Starch and Molasses factory", Borshchino village,
25 Kostroma Region, Russia. The amount of kvas wort concentrate used in the blending mix depends on whether the final mixing step involves the addition of any unfermented kvas wort concentrate. If as discussed below, 95% of the kvas wort concentrate solids are added as unfermented kvas wort concentrate during the final mixing step, then the amount of kvas wort concentrate used in the initial blending
30 step (step ai)) will typically be sufficient to contribute 5% w/w of the solids in the final beverage. Generally, the kvas wort concentrate used in the blending step ai) may be sufficient to contribute from 1-5% of the solids in the final beverage.

Thus as discussed in further detail below, the amount of kvas wort concentrate used in step ai) may be calculated depending on the desired volume of
35 the final beverage, the desired % of wort solids in the final beverage, the wort solids

content of the kvas wort concentrate and the amount of unfermented kvas wort concentrate to be added in the final mixing step. The kvas wort concentrate used in step ai) may typically have a solids content of 68-73% e.g. 70-72%.

5 The sweetener as used in step ai) is as already discussed above, e.g. may be any sugar source. The amount of sweetener used in step ai) may be typically 15% e.g. 5, 10, 15, 20 or 25% of the total amount of sweetener used in the complete method for producing kvas beverage. Alternatively viewed, the amount of sweetener used is calculated based on the target ethanol content of the fermentate after fermentation. As discussed below and previously, it is preferred that the
10 fermentate for this method comprises from 4-13% v/v ethanol. It is possible to calculate from this the amount of sweetener e.g. sucrose required to produce the necessary % ethanol. This is well within the competency of a skilled person. Thus, for example, it is known that in a biochemical reaction 342kg sucrose may be converted into 184kg of ethanol. It is possible therefore to calculate the amount of
15 sweetener required to produce any amount of ethanol. A small excess of sweetener (approximately 10%) may be further included in the reaction since the yeast consume sugar also for metabolic processes other than ethanol production. As discussed further below, sweetener is also generally added during the mixing step for final beverage production for taste consideration, where the amount added
20 is usually within the range of 1-3% w/w of the final product.

Water is also included in the blending step ai) to achieve a kvas wort concentrate with a solids content of 15-40% (preferably 15-34% and even more preferably 19-30%) e.g. 15-35, 15-36, 15-37, 15-38, 15-39, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 16-39, 16-38, 16-37, 16-35, 16-30, 16-29, 16-28, 16-27, 17-39,
25 17-38, 17-37, 17-35, 17-30, 17-29, 17-28, 17-27, 18-39, 18-38, 18-37, 18-36, 18-34, 18-33, 18-30, 18-29, 18-28, 18-27, 19-39, 19-38, 19-36, 19-37, 19-35, 20-39 20-38, 20-37, 20-30, 20-29, 20-28, 20-27, 21-39, 21-38, 21-37, 21-35, 21-30, 21-29, 21-28, 22-39, 22-38, 22-35, 22-30, 22-29, 22-28, 23-38, 23-37, 23-35, 23-30, 23-29, 23-28, 24-39, 24-38, 24-37, 24-30, 24-29, 24-28, 25-39, 25-38, 25-37, 25-36, 25-30%.

30 Particularly, a solids content of 30% may be used. The water quantity can be calculated based on the amount of sweetener, amount of kvas wort concentrate and target solids for the preparation. As discussed previously, the amount of sweetener may be calculated based on required ethanol content of the fermentate and the amount of kvas wort concentrate may be taken at the level of 5% of the

dosage in the final beverage (as the fermentate will constitute 5% of the final beverage).

An example detailing the calculation of kvas wort concentrate required for the fermentation is as follows. Thus, if it is required for taste considerations to have 2% of kvas wort concentrate solids in the final beverage, in 1000kg of final beverage there will be 20kg of solids from the wort concentrate. Additionally, assuming that available kvas wort concentrate contains 70% solids then the actual amount of concentrate is $20/0.7 = 28.6$ kg in the final beverage. As approximately 5% of this amount may be used for the fermentate production, 1.43kg will be required to be blended in step ai) for the fermentation reaction. As discussed further below however, at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 or 100% of the kvas wort concentrate used in the method may be employed in step ai). This method is therefore not limited to the use of only 5% of the total amount of kvas wort concentrate in step ai). Thus, in the case that 100% of the kvas wort concentrate is used in step ai) (and thus no unfermented kvas wort concentrate is added during a later mixing step), 28.6kg of kvas wort concentrate would be used for the blending step in the example provided. To ensure sufficient water activity for yeast survival and propagation the solid content in the preparation should be preferably less than 30%. On the other hand for economic reasons it is desirable to transport not more than 10% of the volume of final product. Setting the target ethanol concentration in the final beverage at 0.5% the target ethanol content in the deep fermentate may be determined at 5% v/v. From this the amount of added sugar and water may be calculated using the method of biochemical reaction described above.

The amount of water added therefore depends on the initial concentration and amount of the kvas wort concentrate and sugar used in the method. The solids content of the kvas wort concentrate may be measured by refractometry and/or densitometry. Preferably, a refractometric method is used because it may be simpler to perform (no dilution is required) and provides technical precision.

Step bi) of the method involves the fermentation of the product of step ai) with yeast to preferably obtain 4-13% v/v ethanol e.g. 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13% v/v ethanol or 4-12, 4-11, 4-10, 4-9, 4-8, 5-12, 5-10, 5-9, 5-8, 6-12, 6-11, 6-10, 6-9, 7-12, 7-11, 7-10, 7-9, 8-12, 8-11, 8-10,% v/v ethanol. Particularly the highest ethanol concentration possible is reached although yeast numbers generally start to decline after 10% ethanol is reached. Ethanol concentration can be measured as

discussed previously above. The yeast which can be used in the fermentation are as already discussed above in relation to method steps a) to e).

The fermentation step may be carried out under the following conditions
Thus, as discussed previously herein, dry yeast may be used in fermentation step
5 bi) where dry yeast may be rehydrated before inoculation. For rehydration,
preferably 10% w/w suspension of dry yeast may be prepared at a temperature of
30°C. Rehydration can be carried out in water solution of fermentable
carbohydrates; or in diluted or undiluted wort; or just in process water. Such a
suspension may be gently mixed to ensure complete rehydration of the yeast. The
10 suspension may be left for at least 10, 20 or 30 minutes (preferably 30 minutes) and
then may be added to the product of step ai). The fermentation may be carried out
in cylindrical-conical vessels typically used in the brewing industry, which allow
aeration. Fermentation step bi) may be particularly carried out at 25-30°C and
more particularly at 28°C and may take from 8 to 48 hours depending on specific
15 conditions.

A deep fermentation step is preferably carried out in step bi), which
generally allows the yeast to utilise all the fermentable sweetener present e.g. at
least more than 90% of the fermentable sweetener may be utilised. The
fermentation process needs to be supported by appropriate aeration. The
20 fermentation may not be artificially terminated until the target ethanol content has
been reached, although termination of the fermentation may be artificially
employed, e.g. by chilling, when the yeast begin to lyse which may result in an
unpleasant taste occurring in the final beverage.

A 4-13% v/v ethanol concentration is obtained by using an appropriate
25 amount of sweetener in blending step ai) (discussed above) to allow the production
of the required ethanol concentration by yeast.

As for the method discussed previously, an additional fermentation step may
also be carried out in the present method using acid producing bacteria, preferably
lactobacteria. Thus, the method may include two fermentation steps, where one
30 fermentation is carried out with bacteria and the other fermentation step is carried
out with yeast. Preferably, parallel fermentation may be carried out in this method,
where one part of the product of step ai) may be subjected to a bacterial
fermentation and the second part of the product of step ai) may be subjected to a
yeast fermentation. In this case, it may be preferable to ferment using acid
35 producing bacteria, preferably lactobacteria, to reach an acidity of 0.2-0.7% w/w

expressed as citric acid and then to concentrate this product to an acidity of 1.5-2% e.g. by evaporation. This fermentate should preferably be pasteurized to ensure at least 5-log reduction of the acid producing bacteria. The products of the yeast and bacterial parallel fermentations may then be mixed together as discussed
5 previously.

Alternatively, if a bacterial fermentation step is not carried out, it is possible to add food acids at the final mixing stage of the kvas beverage. Thus, the fermentation with bacteria, preferably lactobacteria, is an optional step in the production of the fermented kvas wort concentrate which can be avoided by
10 including food acids in the final mixing stage.

The product of the yeast fermentation of step bi) comprises most of the sensorial properties of kvas beverage and only a final mixing step is hence required to produce the final kvas beverage. A separation step (step ci)) should also be carried out prior to mixing to remove yeast and other insoluble matter, as discussed
15 above in relation to step c).

Typically, the final mixing step involves the mixing of the product of step bi) with unfermented kvas wort concentrate, sweetener, water and optionally food acids (if a bacterial fermentation step has not been carried out previously). The unfermented kvas wort concentrate used in any such a mixing step may be the
20 same kvas wort concentrate as was used in step ai) of the method of the invention and is preferably a kvas wort concentrate produced using the same initial grist ingredients as the kvas wort concentrate in step ai) e.g. produced with the same carbohydrate source. However, it is possible to use kvas wort concentrate with slightly different properties e.g. of slightly different solids content at this stage, and
25 there is no requirement that the unfermented kvas wort concentrate used be the same or from the same batch as the kvas wort concentrate used in the method step ai)

The amount of unfermented kvas wort concentrate used in any final mixing step may vary from 95% to 0% of the total amount of kvas wort concentrate used in
30 the method of production. Thus, as discussed previously, if there is required to be 2% kvas wort concentrate solids in the final beverage using kvas wort concentrate of 70% solids, this calculates as 28.6 kg of kvas wort concentrate per 1000kg of final beverage. If 5% of the kvas wort concentrate is used in step ai), 95% of kvas wort concentrate may be used in the final mixing step i.e. 27.17 kg of kvas wort
35 concentrate in this example. However, the final mixing step can require the mixing

of any % of unfermented kvas wort concentrate depending on the % kvas wort concentrate used in step ai). Thus, 95, 90, 80, 70, 60, 50, 40, 30, 20, 10 or 0% of the total amount of kvas wort concentrate used can be mixed in the final beverage production. If all of the required kvas wort concentrate is used in step ai), it is possible that no kvas wort concentrate will be added at the final mixing step. Hence, the amount of unfermented kvas wort concentrate added at this stage is dependent on that added at step ai) (and on the required % kvas wort concentrate solids in the final beverage).

A sweetener is usually mixed at this stage to form kvas beverage. The sweetener may be any sugar source as previously discussed and the amount added is usually 1-3% of the solids amount e.g. 2-3%, determined by the desired sweetness of the final beverage product. Typically, a sugar syrup may be used e.g. of 63-66 Brix, typically 64-65% Brix.

Food acids are only included in the final blending step if an earlier fermentation step with acid producing bacteria, preferably lactobacteria, is not carried out as discussed previously. The comments made above in relation to food acids apply equally here. Thus, food acids which may be added in the final blending step include lactic, malic, acetic, tartaric and/or citric acid.

Preferably, the final mixing step results in the production of a beverage with an ethanol content of approximately 0.5%, e.g. from 0.4 to 1.2%. Particularly, the kvas final beverage may comprise from 1-5% kvas wort concentrate solids, e.g. from 1-2, 1-3, 1-4, 2-5, 2-4, 2-3% and particularly 2%. The mixed product may further be filtered or centrifuged to remove undissolved solids from unfermented kvas wort concentrate used during the mixing step. Filtration may be carried out using a simple bag or cartridge filter and centrifugation may be carried out using an industrial continuous stream centrifuge with a capacity of about 2000-30000 l/hour. The beverage may further be carbonated, pasteurised and bottled.

Kvas final beverage may however be produced by 2 conventional methods of beverage industry. Firstly, the beverage can be mixed directly to single strength (4.5 -10 apparent Brix e.g. 5-10, 6-10, 7-10, 8-10, 5-9, 6-9, 7-9, 5-8, 6-8) as described above. Alternatively, it can be made through intermediate final syrup which includes all ingredients but only a fraction of water. This final syrup may contain 15-50% of solids e.g. 20-40, 20-35, 20-30, 30-40, 35-40%. The syrup may thus be alternatively viewed as a concentrated form of kvas. In this case final syrup may be subjected to filtration or centrifugation treatment as described above. At

bottling final syrup may be mixed with still or carbonated water using special equipment referred to as proportioner. The mixing ratio may vary from 2 to 6 or 2 to 10 volume of water per 1 part of syrup e.g. 3, 4, 5, 6, 7, 8, 9, or 10 volumes of water. The invention, therefore further provides a method for making a syrup which
5 can be diluted with still or carbonated water to form kvas, comprising steps ai) to ei) below to produce a syrup with 15-50% solids content. Syrup or beverage may be additionally treated by bubbling of sterile air to reduce undesirable off-notes.

Thus, the invention also encompasses a method for the production of kvas beverage comprising the steps of :

- 10 ai) blending kvas wort concentrate with sweetener and water to produce kvas wort concentrate with 15- 40%, e.g.15-34%, solids content,
bi) fermenting product of step ai) with yeast to obtain 4-13% v/v ethanol,
ci) separating yeast from product of bi),
di) mixing fermented product of bi) with sweetener, water and optionally food
15 acids and/or unfermented kvas wort concentrate
ei) separating undissolved solids from product of step di).

The water use in mixing step di) is preferably beverage quality water as discussed above.

Further, the invention provides a method for producing a syrup which can be
20 diluted with still or carbonated water to form kvas comprising the steps of

- ai) blending kvas wort concentrate with sweetener and water to produce wort with 15-40%, e.g. 15-34%, solids content,
bi) fermenting product of step ai) with yeast to obtain 4-13% v/v ethanol,
ci) separating yeast from product of bi),
25 di) mixing product of ci) with sweetener, water and optionally unfermented kvas wort concentrate and/or food acids to form a syrup comprising a solids content of 15-50% as measured as refractometric Brix and
ei) separating undissolved solids from product of step di).

The above methods with steps ai) and bi) or ai) to ei) may also include steps
30 of producing the kvas wort concentrate from the prior art method and as already discussed previously.

Thus, the deep fermentate (fermentate comprising 4-13% ethanol) as produced by the methods discussed above can be used to produce kvas beverage.

Alternatively viewed therefore, the present invention provides a method for
35 producing kvas beverage comprising the step of mixing concentrated fermented

kvas wort with sweetener, water and optionally food acids and an aromatic solution comprising at least 20% v/v ethanol.

5 Additionally, the present invention provides a method for producing kvas beverage comprising the step of mixing a fermentate of a wort comprising 4-13% v/v ethanol with sweetener and water and optionally food acids and/or unfermented kvas wort concentrate.

10 Preferably, when said concentrated fermented kvas wort is blended with an aromatic solution comprising at least 20% ethanol, said concentrated fermented kvas wort is obtainable by method steps a) to e) as previously described i.e. by a) blending kvas wort with sweetener, b) fermenting product of a) with yeast to provide an ethanol content of 2.5-5% v/v, c) separating yeast from product of b), d) distilling fermented separated wort product of c) to produce a concentrated wort with a solids content of at least 65% measured as refractometric Brix and e) heat treating wort concentrate.

15 Further, when said deep fermentate is mixed with sweetener, water e.g. beverage quality water and optionally food acids and/or unfermented kvas wort concentrate, preferably said deep fermentate is obtainable by method steps ai) and bi) as described previously i.e. ai) by blending kvas wort concentrate with sweetener and water to produce wort with 15-40%, e.g. 15-34%, solids content and
20 bi) fermenting product of step ai) with yeast to obtain 4-13% v/v ethanol.

The sweetener and water amounts to be mixed in the method of producing a kvas beverage are as already discussed previously for methods involving steps a) to e) and ai) and ci). Further, as already discussed above, the sweetener may be any type of sugar source.

25 As discussed in detail above, food acids may only be required to be added if an additional step of fermentation by acid producing bacteria, preferably lactobacteria, has not been carried out. The amounts of food acids and types of food acids which may be blended to produce kvas beverage have already been discussed above.

30 In a further embodiment of the invention, a concentrated fermented kvas wort obtainable by method steps a) to e) is encompassed, and a fermentate obtainable by method steps ai) to ci) is encompassed.

Additionally, kvas beverage obtainable by method steps a) to f) or by method steps ai) to ei) as discussed above is encompassed.

Typically, the kvas beverage produced by steps ai) to ei) may comprise 9-11% (e.g. 10-11%) of deep fermentate with 4-6% v/v ethanol (e.g. 5% v/v), 6-8% sugar syrup of 64-65 Brix (e.g. 7-8% sugar syrup), 0.06-0.07% Lactic acid 80-90% (e.g. 85-90, 86-89, or 88%), 0.05-0.07% Acetic acid 60-80% (e.g. 65-75% or 70%),
5 syrup water 3-6% (e.g. 4-5%) and beverage water 75-80% (e.g. 77-79%), where the % are % w/w in the final beverage.

Typically the kvas beverage produced by steps ai) to ei) may comprise:

1) volatiles, particularly diacetyl at 100-400, 150-300 or 200-250 ppb, e.g.
10 244 ppb, pentanedione at 30-150, 40-100, 50-60 or 50-80 ppb, e.g. 51 ppb, ethyl acetate at 0.5-5.0, 0.5-1.5, 0.5-1.0, 0.8-1.0, 0.9-1.1, 1.0-1.2, 1.0-1.1, 1.0-1.3 or 1.0-5.0 ppm, e.g. 1.0 ppm, propanol at 3.0-30.0, 5.0-7.0, 6.0-7.0, 6.2-6.8 or 6.6-6.8 ppm, e.g. 6.7 ppm, Isobutanol at 1.0-30.0, 15-17, 13-17, 14-18, 15-20, 15-19 or 16-20 ppm, e.g. 16.2 ppm, Isoamy alcohol at 1.0-30.0, 15-17, 12-16, 13-17, 14-18, 15-
15 20 or 15-19 ppm, e.g. 15.3 ppm, acetaldehyde at 1.0-30.0, 4-6, 5-7, 5-6 or 5-8 ppm, e.g. 5.1 ppm.

2) organic acids, particularly pyruvate at 0.01-1.0, 0.01-0.1, 0.01-0.05, 0.02-0.05, 0.03-0.05 or 0.04-0.06 g/l, e.g. 0.05 g/l, malate at 0.1-5.0, 0.3-0.6, 0.4-0.6 or 0.5-0.8 g/l, e.g. 0.57 g/l, acetate at 0.05-0.5 or 0.1-0.5 g/l, e.g. 0.12 g/l, lactate at
20 0.05-5.0, 0.6-0.8 or 0.6-0.9 g/l, e.g. 0.63 g/l, succinate at 0.05-5.0, 0.5-0.8 or 0.6 g/l, e.g. 0.64 g/l.

3) fermentable sugars, particularly fructose at 0.05-5.0, 2.0-2.1 or 2.1-2.2 g/l, e.g. 2.1 g/l, glucose at 0.05-5.0 or 2.2-2.3 g/l, e.g. 2.3 g/l, saccharose at 10-100, 2-80, 30-60, 45-46 or 46-47 g/l, e.g. 46.0 g/l, maltose at 0.05-15.0, 1.0-10.0,
25 3.0-7.0, 5.8-5.9 or 5.9-6.0 g/l, e.g. 5.9 g/l.

Typically the kvas beverage produced by steps ai) to ei) may have the following physical properties: density of 0.1-5.0 g/cm³, e.g. 1.0248 g/cm³, alcohol of 0.1-1.2 %v/v, e.g. 0.54 %v/v, alcohol of 0.1-1.0 %w/w, e.g. 0.42%w/w, apparent extract 1.0-10.0 °P, e.g. 6.74°P, real extract 1.0-10.0 °P, e.g. 6.94 °P).

30 In a particularly preferred embodiment the kvas beverage produced by steps ai) to ei) comprises 9-11% of deep fermentate with 4-6% v/v ethanol, 6-8% sugar syrup of 64-65 Brix, 0.06-0.07% Lactic acid 80-90%, 0.05-0.07% Acetic acid 60-80%, syrup water 3-6% and beverage water 75-80%, where the % are % w/w in the final beverage.

The invention will now be described in more detail in the following non-limiting Examples with reference to the drawings in which:

5 Figure 1 shows a flow chart demonstrating the prior art industrial process for kvas manufacturing. Steps include cereal cooking, mashing, filtration, and concentration/cooking to produce kvas wort concentrate. Kvas wort concentrate is then blended and fermentation of the kvas preparation is carried out to produce final kvas beverage.

10 Figure 2 shows a flow chart demonstrating the entire production method for kvas beverage of the present invention. Thus, production of kvas wort is the same as in the prior art method. However, kvas wort is subjected to fermentation prior to the final blending step for kvas beverage. Further, distillation of the fermentation product allows the formation of a concentrate and distillate which can be blended at a later time to form kvas beverage.

15 Figure 3 shows a flow chart demonstrating the production method for kvas beverage of the present invention. Production involves blending kvas wort concentrate with sugar/glucose syrup and water and fermentation with yeast to produce a product with 4-13% ethanol content. This product can finally be blended with unfermented kvas wort concentrate, sugar/glucose syrup, water and optionally food acids to form kvas beverage.

20 Figure 4.1 shows a flow chart demonstrating an example blending process of the present invention. Kvas wort concentrate (KWC) is blended with sugar/glucose syrup and water and then pasteurised. Sterile air and yeast are then added to the pasteurised blend in the fermentation vessel.

25 Figure 4.2 shows a fermentation diagram. This graph shows the change in Brix, pH and ethanol levels in the blend over fermentation time.

Figure 4.3 shows a flow chart demonstrating the filling process. The deep fermentate may be further pasteurised before being filled in aseptic Bag-in-Box (BIB) containers are filled.

30 Figure 4.4 is a picture of aseptic filling of the BIB containers which can hold the deep fermentate.

Examples

Example 1: Laboratory batch produced by distillation method

35 Materials:

Single strength (heavy) frozen kvas wort made from fermented rye malt, barley malt, and rye flour with densitometric Brix 16.8

Granulated Sugar cat.1 EC

5 Dry Yeast Safkvas C-79 (supplier Fermentis division of LeSaffre group, France)

Lactic Acid 88%, food grade, (Purac, Netherlands)

Acetic Acid, glacial, food grade (Merck, Germany)

Beverage quality water

a) Blending

10 Calculation of target ethanol content

Assumption is made that sucrose is preferred nutrient for yeast. Initial quantity of heavy wort is 4000 g. This wort contains $4000 \times 0.168 = 672$ g of wort solids. Regular kvas beverage which is a target of this experiment contains 26.51 g of wort solids per 1 l. Therefore from 4000 g of heavy wort $672/26.51 = 25.35$ l of final kvas beverage may be produced. The target ethanol content in commercial kvas product is 0.5 % v/v therefore the total amount of ethanol to be produced by yeast fermentation is $25.35 \times 0.5\% = 0.128$ l. Assuming density of ethanol as 0.789 kg/l, recalculate volume to weight: $0.128 \times 0.789 = 0.1$ kg = 100 g. In biochemical reaction 342 g of sucrose is converted into 184 g of ethanol, therefore to produce 100 g of ethanol $(100 \times 342/184) = 185.9$ g of sugar is to be added to the wort. Carbon dioxide is also produced by the same biochemical reaction in which for 184 g of ethanol 176 g of carbon dioxide is produced, therefore for 100 g of ethanol 95.7 g of carbon dioxide is to be produced. Here it is assumed that practically all this carbon dioxide is removed from fermentate during separation process and therefore the weight of carbon dioxide is subtracted from total weight of fermentate. The calculated concentration of ethanol in fermentate will be therefore $100/(4000 + 185.9 - 95.7) = 2.44\%$ w/w. Without accounting of carbon dioxide the concentration would be $100/(4000 + 185.9) = 2.39\%$ w/w. It is possible to conclude therefore that for technical purpose carbon dioxide weight may be neglected. After addition of sugar calculated solids in preparation is $(672 + 185.9)/(4000 + 185.9) = 20.49\%$. Measured solids as densitometric Brix are 20.63., measured apparently density 1.08268 kg/l.

Summary of blend composition is shown in Table 1.1:

Ingredient	Weight, g	Volume, ml	Density g/ml
Heavy wort	4000		

Sugar	185.9		
Total	4185.9	3867	1.08268

b) Fermentation

Due to volume limitation of experimental flask 3600 g of blend was inoculated with 5.5 g of dry yeast Safkvas C-79. According to supplier specs the content of living cells is $6 \cdot 10^9$ per g of dry yeast. The pitching rate is therefore $5.5 \cdot 6 \cdot 10^9 / 3600 = 9.2 \cdot 10^6$ cell/g. The flask was closed with gas transparent stopper. Flask was incubated at 28°C under continuous shaking. Material was not aerated but was continuously shaken during fermentation.

Fermentation trial is summarized in Table 1.2, depending on fermentation time. F2 is the value of the fermentate after centrifugation, just before distillation.

Table 1.2: Fermentation parameters

Parameter	F1	F2
	(17h)	
Density (g/cm ³)	1.05036	1.04399
Alcohol (%v/v)	4.17	4.37
Alcohol (%w/w)	3.13	3.30
Apparent extract (°P)	12.93	11.42
Apparent attenuation (%)	14.35	12.92
Original extract (°P)	20.16	19.07

c) Centrifugation

Fermentate is centrifuged at 4°C and harvested in plastic flask (F2)
Density (Plato degree (gr solids/100gr wort), extracts & ethanol are measured with Anton Paar Beer Alcoyzer combined with density meter DMA 4500.

d) Distillation

Whole volume is distilled, residue (R) and distillate (D) fractions are measured for ethanol content. Volatile organic compounds (VOC), corresponding to the organoleptic profile of the distillate is measured by headspace chromatography (GC-HS). Distillation trial is summarized in Table 1.3 (final point). Organoleptic profile of the distillate fraction is listed on Table 1.4.

Table 1.3: Distillation parameters

Parameter	Residue	Distillate Diluted 5x
Density (g/cm ³)	1.06426	0.99005
Extract (°P)	16.86	N/A
Alcohol (%v/v)	0.38	5.76 (x5 = 28.8)
Alcohol (%w/w)	0.28	4.59 (x5 = 22.9)

Table 1.4: Distillate organoleptic profile

Compound	Distillate
Diacetyl (ppm)	3.5
Pentanedione (ppm)	2.7
Total VDK (ppm)	6.2
Ethyl acetate (ppm)	21.7
Isoamyl acetate (ppm)	1.1
Total esters (ppm)	22.8
Propanol (ppm)	67.9
Isobutanol (ppm)	155.9
Isoamyl alcohol (ppm)	609.8
Total fusel alcohol (ppm)	833.6
Acetaldehyde (ppm)	54.4

e) Concentration

Residue was concentrated using typical laboratory glass distillation system.

- 5 Concentration trials are summarized in Table 1.5. Organoleptic profile of the evaporate fraction is listed on Table 1.6.

Table 1.5: Concentration parameters

Parameter	Evaporate	Concentrate Diluted 5x
Density (g/cm ³)	0.99752	1.05424
Extract (°P)	0.79	14.23(x5 = 71.1)
Alcohol (%v/v)	0.48	0.21
Alcohol (%w/w)	0.38	0.16

Table 1.6: Evaporate organoleptic profile

Compound	Evaporate
Diacetyl (ppb)	122.2

Pentanedione (ppb)	21.5
Total VDK (ppb)	143.7
Ethyl acetate (ppm)	-
Isoamyl acetate (ppm)	-
Total esters (ppm)	-
Propanol (ppm)	6.6
Isobutanol (ppm)	8.7
Isoamyl alcohol (ppm)	20.4
Total fusel alcohol (ppm)	35.7
Acetaldehyde (ppm)	2.1

The latter data show that most of volatiles (about 90%) are kept in disillate fraction. As shown in Table 1.5. Fermented concentrate contains 71.1% solids measured as densitometric Platto.

5 **f) Final heat treatment of fermented concentrate**

Fermented concentrate was later heat treated in closed glass bottle in Fedegari autoclave at 120°C 2 times for 5 min. Product was rigorously shaken between treatment cycles.

10 **g) Mixing of 3 l final beverage.**

Beverage is made through final syrup stage. Final syrup is diluted with carbonated water with ratio 1 to 2.

15 Calculation of quantity of distillate. As stated above the target ethanol content in final beverage is 0.5 %v/v. To achieve this in 3 l it is necessary to add 52.1 ml of distillate with 28.8% v/v of ethanol. With density of distillate 0.99005 kg/l, the weight of added distillate is 51.6 g

Calculation of quantity of fermented concentrate. As stated above the target quantity of wort solid in final beverage is 26.51 g per 1 l. For 3 l it is needed to add 111.9 g of fermented wort concentrate with 71.1 % solid.

20 Quantity of glacial acetic acid is taken directly from regular beverage formula, beverage acidity adjusted to target by lactic acid 88%.

Finally target beverage solids as apparent Brix are adjusted by added sugar.

Summary beverage formula is shown in Table 1.7.

Ingredient	Weight (g)	Volume (ml)	Density g/ml
------------	------------	-------------	--------------

Distillate	51.6	52.1	0.99005
Fermented wort concentrate	111.9	87.7	1.35308
Sugar	119.9		
Lactic acid 88%	2.7	2.23	1.21
Acetic acid glacial	1.25	0.65	1.16
Syrup water	788.0		
Beverage water	1994.3		
Total	3069.7	3000.1	1.02321

Example 2: Production of laboratory batch using deep fermentation method with 5% of kvas wort concentrate is deep fermentate

Materials:

5 Kvas wort concentrate made from fermented rye malt, barley malt and corn flour with 70.1% solids measured as refractometric Brix and acidity 1.00% w/w as citric acid

Granulated Sugar cat.1 EC

10 Dry Yeast Saffkvas C-79 (supplier Fermentis division of LeSaffre group, France)

Lactic Acid 88%, food grade, (Purac, Netherlands)

Acetic Acid, glacial, food grade (Merck, Germany)

Beverage quality water

15 **ai) Blending**

Calculation of lab batch

This deep fermentate should provide all ethanol in final beverage. Since only 5% of kvas wort concentrate is used in fermentate, it should constitute about 5% of final product. Setting target ethanol content in final beverage 0.5% v/v, it is possible to calculate target ethanol in deep fermentate as 10% v/v.

20 About 3 l of blend needs to be prepared for fermentation to meet fermentation flask volume and analytical needs. The target content of ethanol is therefore 0.3 l or 0.237 kg assuming density as 0.789 kg/l.

25 In biochemical reaction 342 g of sucrose is converted into 184 g of ethanol, therefore to produce 237 g of ethanol $(237 \times 342 / 184) = 440.5$ g of sugar is to be

added to the blend. For this experiment it was decided to use 20% surplus of sugar, 530 g was actually added so. Due to composition of kvas wort concentrate used in this experiment final beverage should contain 35.14 kg of wort solids per 1000 l. Therefore 5% of this amount should be used in blend for deep fermentation:

5 35.14*0.05=1.78 kg per 1000 l of final beverage. 3l of deep fermentate should suffice to produce 60 l of final beverage. Therefore for 60 l of final beverage 107 g of solids or 152 g of kvas wort concentrate of 70.1% solids is needed.

Total amount of solids in blend is 530+107=637 g. To achieve comfortable condition for fermentation solid content should not exceed 20%. The total weight of blend is therefore 637/0.2=3183 g. The added water is therefore 3183-682=2501 g. 10 Then water was increased up to 2800 g.

Summary of blend composition is shown in Table 2.1:

Ingredient	Weight, g	Volume, ml	Density g/ml
Kvas wort concentrate	152		
Sugar	530		
Water	2800		
Total	3482	3247	1.07232

Calculated solids is 18.28%

15 Solids measured as refractometric Brix 18.24

Based on calculation in previous example carbon dioxide formation is neglected in material balance.

bi) Fermentation

2251 g of blend was inoculated with 28 g of dry yeast Safkvas C-79.

20 According to supplier specs the content of living cells is $6 \cdot 10^9$ per g of dry yeast. The pitching rate is therefore $28 \cdot 6 \cdot 10^9 / 2251 = 75 \cdot 10^6$ cell/g. The flask was closed with gas transparent stopper. Flask was incubated at 28 C under continuous shaking. Material was not aerated but was continuously shaken during fermentation. Fermentation trial is summarized in Table 2.2, depending on 25 fermentation time.

Table 2.2: Fermentation parameters

Parameter	F1 (22h)	F2(26h)	F3 (43h)	F4 end
Density (g/cm ³)	1.02953	1.02108	0.99688	0.99676

Alcohol (%v/v)	6.02	7.10	10.27	10.33
Alcohol (%w/w)	4.61	5.49	8.13	8.18
Apparent extract (°P)	7.91	5.81	0.00	0.00
Real extract (°P)	9.98	8.26	3.20	3.18
Real attenuation (%)	48.87	57.86	84.02	84.15
Apparent attenuation (%)	57.49	68.60	100.00	100.00
Original extract (°P)	18.60	18.52	18.40	18.47

The final ethanol content is 10.33%v/v in 43 hours.

ci) Centrifugation

Deep fermentate is centrifuged at 4°C and harvested in plastic flask (F4)

- 5 Density (Plato degree (gr solids/100gr wort) , extracts & ethanol are measured with Anton Paar Beer AlcoLyzer combined with density meter DMA 4500.

Deep fermentate was analysed for volatile organoleptic compounds (VOC) profiles of sugars and organic acids.

- 10 VOC are measured by headspace gas chromatography (GC-HS) Organoleptic profile of deep fermentate is presented in Table 2.3:

Table 2.3 : Organoleptic profile of deep fermentate

Compound	
Diacetyl (ppb)	46
Pentanedione (ppb)	35
Total VDK (ppb)	81
Ethyl acetate (ppm)	19.6
Isoamyl acetate (ppm)	0.73
Total esters (ppm)	20.3
Propanol (ppm)	10.5
Isobutanol (ppm)	60.3
Isoamyl alcohol (ppm)	390.1
Total fusel alcohol (ppm)	460.9
Acetaldehyde (ppm)	109.5

Organic acids profile was measured by HPLC. Organic acids content in deep fermentate is presented in Table 2.4:

Table 2.4: Organic acids in deep fermentate

Acids (g/l)

- 37 -

Pyruvate	0.06
Malate	1.05
Succinate	2.01

Sugars profile of deep fermentate was measured by HPLC. Sugar profile of Kvas is presented in Table 2.5

Table 2.5: Sugar profile

Sugar (g/l)	
Fructose	0.00
Glucose	0.25
Saccharose	0.11
Maltose	2.50
Maltotriose	1.53
Total fermentable sugars	4.39
Total fermentable sugars (%)	0.44

This analysis proves initial assumption that sucrose is preferred nutrient for yeast in this matrix is confirmed. It also proves that carbohydrates containing in kvas wort concentrate are hardly consumed by yeast.

di) Mixing of 3 l final beverage

Beverage is made through final syrup stage. Final syrup is diluted with carbonated water with ratio 1 to 2. Syrup is centrifuged to remove solid particles of unfermented kvas using lab centrifuge at 5000 rpm. Syrup was also bubbled with sterile air using lab compressor with sterile filter at inlet for 20 min.

Calculation of quantity of deep fermentate: As stated above deep fermentate constitutes 5% of volume of final beverage For 3 l it is necessary to add 150 ml of deep fermentate with 10.33% v/v of ethanol. With density of distillate 0.99676 kg/l, the weight of added deep fermentate is 149.5 g. The concentration of ethanol in final beverage is therefore 0.52% v/v. The amount of kvas wort concentrate solids in 149.5 g of deep fermentate is 4.57 g.

Calculation of quantity of kvas wort concentrate: As stated above the target quantity of wort solid in final beverage is 35.14 g per 1 l. For 3 l it is needed to add 105.4 g of fermented wort concentrate solids. Since 4.57 g of solids is added with deep fermentate the rest is 100.8 g, which is equivalent to 143.9 g of kvas wort concentrate.

Quantity of glacial acetic acid is taken directly from regular beverage formula, beverage acidity adjusted to target by lactic acid 88%.

Finally target beverage solids as apparent Brix are adjusted by added sugar.

Summary beverage formula is shown in Table 2.6.

Ingredient	Weight (g)	Volume (ml)	Density g/ml
Deep fermentate	149.5	150	0.99676
Kvas wort concentrate	143.9		
Sugar	99.1		
Lactic acid 88%	2.2		
Acetic acid glacial	1.25		
Syrup water	679.7		
Beverage water	1994.7		
Total	3070.35	3000.7	1.02321

5 Product manufactured with this method was analytically compared with product manufactured with conventional technology. Results are shown in Table 2.7

Table 2.7 Analytical comparison of new and conventional technologies

Compound	Deep fermentate	Final product	Control product
Diacetyl (ppb)	46	5.65	4.99
Pentanedione (ppb)	35	6.26	7.06
Ethyl acetate (ppm)	19.6	Not quantified	Not quantified
Isoamyl acetate (ppm)	0.73	Not quantified	Not quantified
Propanol (ppm)	10.5	0.04	0.45
Isobutanol (ppm)	60.3	2.08	Not quantified
Isoamyl alcohol (ppm)	390.1	18.68	17.29
Acetaldehyde (ppm)	109.5	0.99	3.66

10 **Example 3: Production of laboratory batch using deep fermentation method with 100% of kvas wort concentrate is deep fermentate**

Materials:

Kvas wort concentrate made from fermented rye malt, barley malt and rye flour with 71.5% solids measured as densitometric Brix and acidity 2.15% w/w as citric acid

15

- Granulated Sugar
- Dry Yeast (supplier JCS Enzym, Ukraine)
- Lactic Acid 88%, food grade, (Purac, Netherlands)
- Acetic Acid, glacial, food grade (Ukraine)
- 5 Beverage quality water

ai) Blending

Calculation of lab batch of 3 l

10 This deep fermentate should provide all ethanol in final beverage. For economic reason it is assumed that volume of deep fermentate should not exceed 10% of final beverage volume. Therefore from 3 l of deep fermentate 30 l of final beverage should be produced. Due to composition of kvas wort concentrate used in this experiment final beverage should contain 36.55 kg of kvas wort concentrate per 1000 l.

15 Therefore 30 l of final beverage should contain $36.55 \times 30 / 1000 = 1.1$ kg of kvas wort concentrate.

Setting target ethanol content in final beverage 0.5% v/v, it is possible to calculate target ethanol in deep fermentate as 5% v/v. The target content of ethanol is therefore 0.15 l or 0.118 kg assuming density as 0.789 kg/l.

20 In biochemical reaction 342 g of sucrose is converted into 184 g of ethanol, therefore to produce 118 g of ethanol $(118 \times 342 / 184) = 219$ g of sugar is to be added to the blend. For this experiment 7.8% surplus of sugar, thus 236 g was actually added.

25 Total amount of solids in blend is $236 + 1100 \times 71.5\% = 1022.5$ g. It was decided to test blend with 30% solids content The total weight of blend is therefore $1022.5 / 0.3 = 3408$ g. The added water is therefore $3408 - 236 - 1100 = 2072$ g.

Summary of blend composition is shown in Table 3.1:

Ingredient	Weight, g	Volume, ml	Density g/ml
Kvas wort concentrate	1100 g		
Sugar	236 g		
Water	2072		
Total	3408	3027	1.12594

Solids measured as densitometric Brix 29.69

Based on calculation in previous example carbon dioxide formation is neglected in material balance.

bi) Fermentation

3408 g of blend was inoculated with 20 g of dry yeast CJSC Enzym.

5 According to supplier specs the content of living cells is $8 \cdot 10^9$ per g of dry yeast. The pitching rate is therefore $20 \cdot 8 \cdot 10^9 / 3408 = 47 \cdot 10^6$ cell/g. The flask was closed with gas transparent stopper. Flask was incubated at 28 C under continuous shaking. Material was not aerated but was continuously shaken during fermentation. Fermentation trial is summarized in Table 3.2, depending on
10 fermentation time.

Table 3.2: Fermentation parameters

Parameter	F1 (2h)	F2(6h)	F3 (8h)	F4 end
Alcohol (%v/v)	0.71	2.56	4.83	5.76
Apparent extract (°P)	28.7	25.32	21.25	19.62
Cell count (visual assessment)	$128 \cdot 10^6$			

The final ethanol content is 5.76%v/v.

ci) Separation

15 Deep fermentate is separated using laboratory filtration apparatus with paper filter equivalent to Whatman 114v into glass flask (F4)
Plato degree (gr solids/100gr wort) , extracts & ethanol are measured with Anton Paar Beer Alcoalyzer combined with density meter DMA 4500.

di) Mixing of 3 l final beverage

20 Beverage is made through final syrup stage. Final syrup is diluted with carbonated water with ratio 1 to 4.

Calculation of quantity of deep fermentate: As stated above deep fermentate constitutes 10% of volume of final beverage For 3 l it is necessary to add 300 ml of
25 deep fermentate with 5.76% v/v of ethanol. With density of distillate 1.07823 kg/l, the weight of added deep fermentate is 324 g. The concentration of ethanol in final beverage is therefore 0.576% v/v.

Quantity of glacial acetic acid is taken directly from regular beverage formula, beverage acidity adjusted to target by lactic acid 88%.

30 Finally target beverage solids as apparent Brix are adjusted by added sugar.

Summary beverage formula is shown in Table 3.3.

Ingredient	Weight (g)	Volume (ml)	Density g/ml
Deep fermentate	324	300	1.07823
Sugar	133.1		
Lactic acid 88%	2.13		
Acetic acid glacial	1.25		
Syrup water	215.2		
Beverage water	2394		
Total	3069.68	3000.05	1.02321

Example 4: Production of industrial batch using deep fermentation method with 100% of kvas wort concentrate converted into deep fermentate

5 The purpose of experiment was to assess full supply chain of new method and test final product against conventional competitive product.

Materials:

10 Kvas wort concentrate made from fermented rye malt, barley malt and rye flour with 71.5% solids measured as densitometric Brix and acidity 1.58% w/w as citric acid (supplier JSC ATRUS, Russia)

Granulated Sugar

Dry Yeast (supplier JCS Enzym, Ukraine)

Lactic Acid 88%, food grade, (Purac, Netherlands)

15 Acetic Acid, glacial, food grade (supplier CJSC Severodonetskoye ob'edineniye Azot, Ukraine)

Beverage quality water

a) Blending

20 At this phase 40000 l batch was blended in 100000 l industrial cylindrical-conical tank

Calculation of industrial batch of 40000 l

As explained in previous examples this deep fermentate should provide all ethanol in final beverage. For economic reason it is assumed that volume of deep
25 fermentate should not exceed 10% of final beverage volume. Therefore from 40000 l of deep fermentate 400000 l of final beverage should be produced. Due to

- 42 -

composition of kvas wort concentrate used in this experiment final beverage should contain 26.51 kg of kvas wort solids per 1000 l of final beverage. Therefore 400000 l of final beverage should contain $26.51 \times 400000 / 1000 = 10604$ kg of kvas wort solids. Setting target ethanol content in final beverage 0.5% v/v, it is possible to calculate target ethanol in deep fermentate as 5% v/v. The target content of ethanol is therefore $400000 \times 5\% = 2000$ l or 1578 kg assuming density as 0.789 kg/l. In biochemical reaction 342 g of sucrose is converted into 184 g of ethanol, therefore to produce 1578 kg of ethanol ($1578,000 \times 342 / 184$)= 2933 kg of sugar is to be added to the blend. For this experiment about 5% surplus of sugar was used, thus 3100 kg was actually programmed. In industrial process sugar syrup is used. Brix of available syrup was 64.64. Therefore calculated amount of syrup is $3100 / 0.6464 = 4796$ kg.

Due to specifics of industrial blending technology kvas wort concentrate needs to be pre-diluted to reduce density and viscosity. For this production it was diluted to 59.2% solids and 1.3% acidity respectively. The amount of diluted kvas wort concentrate is adjusted in accordance with dilution: $10604 / 0.592 = 17792$ kg. Total amount of solids in blend is $3100 + 10604 = 13704$ kg. It was decided to test blend with 30% solids content. The total weight of blend is therefore $13704 / 0.3 = 45680$ kg. The added water is therefore $45680 - 17792 - 4796 = 23092$ kg. Summary of calculated blend composition is shown in Table 4.1:

Ingredient	Weight, kg	Volume, l	Density kg/l
Kvas wort concentrate (59.2 %)	17792		
Sugar Syrup	4796		
Water	23092		
Total	45680	40571	1.12594

Based on calculation in previous example carbon dioxide formation is neglected in material balance.

See sketch of blending process is shown on Figure 4.1

25

Due to limitation of dosing system accuracy actually dosed quantities were different from calculated as follows:

Summary of actual blend composition is shown in Table 4.2:

Ingredient	Weight, kg	Volume, l	Density kg/l
Kvas wort concentrate (59.2 %)	17491		
Sugar Syrup	4788		
Water	24100		
Total	46379	41368	1.12114

Actual measured Brix was 28.99

5 **b) Fermentation**

46379 kg of blend was inoculated with 180 kg of rehydrated yeast (*Saccharomices cerevesiae*) supplied by CJSZ Enzym. According to supplier specs the content of living cells is 8×10^9 per g of dry yeast. The calculated pitching rate is therefore $180000 \times 8 \times 10^9 / 46379 = 31 \times 10^6$ cell/g. Dry yeast was rehydrated in separate vessel in 1500 kg sugar syrup of 8.02 Brix. Sugar and water were taken from blend components and blending program were adjusted accordingly. Rehydrated yeast suspension was injected into blend in-line. Just before injection of yeast blend was aerated in line with sterile air to achieve oxygen content 6.2 ppm. The amount of added cells was also assessed microscopically just after inoculation and found at 20×10^6 cells/ml. Fermentation diagram is shown in Figure 4.2

The amount of cell was assessed microscopically after 8 hours of fermentation and was found at 210×10^6 cell/ml.

c) Separation

Yeast separation was initiated when ethanol content in deep fermentate reached 5.38% v/v. Separation was undertaken using industrial beer separator GEA Westphalia at fermentation temperature (28-29 C). The amount of residual cells after separation was determined at 15000 per ml. After yeast separation deep fermentate was immediately chilled down to 8 C and pumped into storage tank. The final measurement taken after separation found ethanol at 5.57% and apparent solids at 19.05 Brix.

d) Packaging of deep fermentate for delivery to bottling plant

1000 l Aseptic Bag-in-Box supplied by Scholle is selected as transport package for deep fermentate. There two key advantages of this package for this particular application: it is relatively light and does not require return from customer back to producer. Both are very important for long distance delivery involving customs clearance.

Since deep fermentate after separation contains limited amount of residual yeast pasteurisation step is needed to ensure long term microbiological stability. The following condition is selected for pasteurisation : 96-99 C for 30 sec. Pasteurisation is undertaken with Alpha-Laval equipment. To avoid excessive foaming at filler silicon-base antifoam is added to deep fermentate before pasteurisation step. The sketch of filling part is shown on Figure 4.3. Aseptic filling of BIB is shown on Figure 4.4

The batch was finally packed in 42 1000l BIBs, 38 of which were released for shipping to final bottling plant. 4 were rejected due to various packaging defects.

e) Mixing of 53000 l final beverage

Beverage is made through final syrup stage. Final syrup is diluted with carbonated water with ratio 1 to 4. Due to limitation of syrup tank capacity it was not possible to convert all available 38 BIBs into one batch of final syrup. For the purpose of example only one batch is selected, all others are produced with the same method. 6 BIBs with the following net weight were selected for the first batch production.

Table 4.3: Net weight of selected BIBs, kg

Container #	1	2	3	4	5	6	Total weight
Net weight, kg	1076.25	1076.25	817.95	1076.25	871.76	817.95	5736.41

Table 4.4 Physical properties of deep fermentate

Apparent Brix	Ethanol, %v/v	Density, kg/l	Total volume, l	Total weight, kg	Acidity, %w/w citric
19.10	5.30	1.07651	5328.71	5736.41	0.575

In order to comply with basics of the method deep fermentate should be diluted 10 times by volume. Therefore beverage batch volume should be 53287.1 l and concentration of ethanol should be 0.53% v/v. Other physical properties should be the same as in regular beverage formula.

5 Table 4.5 Physical properties of beverage

Apparent Brix	Ethanol, %v/v	Density, kg/l	Total volume, l	Total weight, kg	Acidity, %w/w citric
6.6	0.53	1.02321	53287.1	54523.90	0.142

Physical properties of final syrup are calculated by application of water/syrup ratio 4.0:1

Table 4.6 Physical properties of final syrup

Apparent Brix	Ethanol, %v/v	Density, kg/l	Total volume, l	Total weight, kg	Acidity, %w/w citric
29.44	2.65	1.12325	10657.4	11970.95	0.647

10 In this production 70% acetic acid was used instead of glacial acetic acid used in the laboratory, therefore its quantity was adjusted and water rebalanced. Syrup acidity was adjusted to target by lactic acid 88%.

Finally target syrup solids as apparent Brix were adjusted by added sugar syrup of 64.45 Brix.

15 Summary of syrup and beverage formulae is shown in Table 4.7

Ingredient	Weight (kg)	Volume (l)
Deep fermentate	5736.41	5328.71
Sugar syrup (64.45 Brix)	3861.12	
Lactic acid 88%	36.95	
Acetic acid (70%)	31.74	
Syrup water	2304.73	
Beverage water	42552.95	
Total	54523.90	53287.1

Final beverage was pasteurized and carbonated before bottling.

Composition of final product

Final beverage was subjected to standard set of analytical assessment consisting of physical properties measurement and volatiles, organic acids and fermentable sugars profiling. The results are shown in the following tables:

5 Table 4.8: Measured physical properties of final beverage

Density (g/cm ³)	1.02480
Alcohol (%v/v)	0.54
Alcohol (%w/w)	0.42
Apparent extract (°P)	6.74
Real extract (°P)	6.94

Table 4.9: Volatiles profile in final beverage

Compound	Value
Diacetyl (ppb)	244
Pentanedione (ppb)	51
Total VDK (ppb)	295
Ethyl acetate (ppm)	1.0
Isoamyl acetate (ppm)	0.0
Total esters (ppm)	1.0
Propanol (ppm)	6.7
Isobutanol (ppm)	16.2
Isoamyl alcohol (ppm)	15.3
Total fusel alcohol (ppm)	38.2
Acetaldehyde (ppm)	5.1

Table 4.10: Organic acids profile in final beverage

Organic acids (g/l)	Value
Pyruvate	0.05
Malate	0.57
Citrate	-
Acetate	0.12
Lactate	0.63
Succinate	0.64

Table 4.11: Fermentable sugars profile in final beverage

Sugar (g/l)	Value
Fructose	2.1
Glucose	2.3
Saccharose	46.0
Maltose	5.9
Maltotriose	-
Total fermentable sugars	56.3
Total fermentable sugars (%)	5.6

This data are very important for assessment of consumers test discussed below.

5 **Consumer test with new product**

This batch of product was tested with consumer by Synovate-Comcon agency in Russia. The product (denominated in test as New-3) outperformed regular commercial version and major competitors.

Consumers were offered the following scale to rate their liking of the products' attributes:

10

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

For statistical assessment, scores were clustered as follows:

Top-2 % of consumers rated the attribute 8 or 9;

Top-3 % of consumers rated the attribute 7, 8 or 9;

15 Top-4 % of consumers rated the attribute 6, 7, 8 or 9.



The summary of key attributes (Taste, Flavour intensity, Flavour naturalness, Aftertaste and Texture (referred below as density)) evaluation is shown in Table 4.7 below. The product described in this example is denominated New-3 K&B.

20

Table 4.7: Summary of key attributes

		New-3 K&B	Current K&B	Rusky Dar	Ochakovo	Nikola
Taste liking	Top-2, %	68	57	59	59	68
	Top-3,%	89	82	81	84	81
	Top-4,%	96	92	93	92	88
	mean (9pt)	7.9	7.5	7.5	7.6	7.5
Flavour intensity liking	Top-3,%	89	78	82	74	83
	Top-4,%	95	91	91	89	87
	mean (9pt)	7.9	7.4	7.5	7.3	7.5
Flavour naturalness	Top-3,%	85	71	74	77	81
	Top-4,%	96	82	88	87	86
	mean (9pt)	7.8	7.2	7.3	7.3	7.4
Aftertaste liking	Top-3,%	84	80	81	81	80
	Top-4,%	94	86	87	90	87
	mean (9pt)	7.9	7.4	7.5	7.5	7.4
Density liking	Top-3,%	86	78	76	75	83
	Top-4,%	95	86	89	84	89
	mean (9pt)	7.9	7.3	7.5	7.3	7.5

5 Base: 150 respondents per New-3 K&B and 120 respondents per each of others

 New-3 K&B is higher at 0.95 sig. level
  New-3 K&B is on par

10 All formulas are rather tasty, the New-3 is better than the current one and competitors for absolute majority of key taste indicators

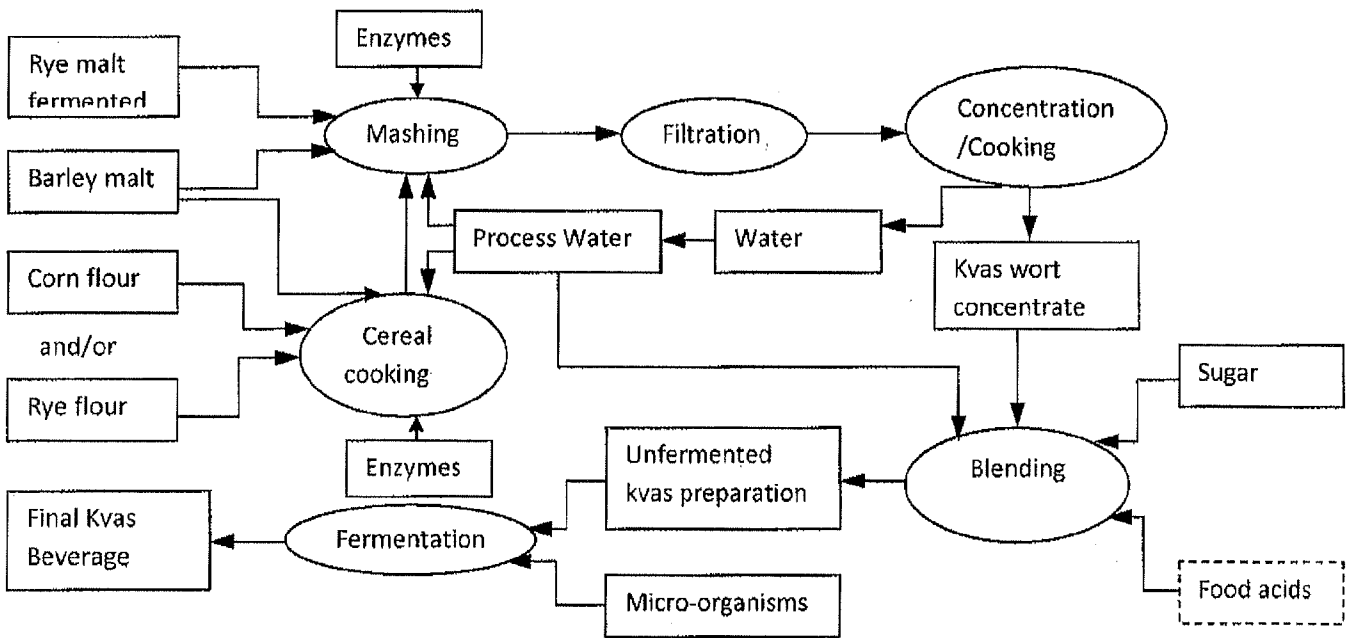
Claims

1. A method for the manufacture of a fermented product, which is capable of being mixed with at least sweetener and water to form kvas beverage comprising the steps of either:
- 5
- a) blending kvas wort with sweetener,
 - b) fermenting product of a) with yeast to provide an ethanol content of 2.5%-5% v/v
 - c) separating yeast from product of b),
 - 10 d) distilling fermented separated wort product of c) to produce a concentrated wort with a solids content of at least 65% measured as refractometric Brix and
 - e) heat treating wort concentrate of d); or
 - ai) blending kvas wort concentrate with sweetener and water to produce
 - 15 wort with 15-40% solids content measured as refractometric Brix,
 - bi) fermenting product of step ai) with yeast to obtain 4-13% v/v ethanol and
 - ci) separating yeast from product of bi).
2. The method of claim 1 wherein the sweetener is sugar, glucose syrup
- 20 and/or fructose syrup.
3. The method of claim 1 or 2 which further comprises a fermentation step with bacteria, preferably lactobacteria, either after step a) but before step d) or after step ai).
- 25
4. The method of claim 3 wherein said fermentation with bacteria achieves an acidity of 0.2-0.7% w/w.
5. The method of any one of claims 1 to 4 wherein said yeast are
- 30 *Saccharomyces cerevisiae*.
6. The method of any one of claims 1 to 5 wherein separation steps c) and ci) are carried out by filtration or centrifugation.

7. The method of anyone of claims 1 to 6 wherein an aromatic solution comprising at least 20% v/v ethanol is obtained during distillation step (d).
8. A method for producing kvas beverage or a syrup comprising 15-50% solids content measured as refractometric Brix which is capable of being diluted with still or carbonated water to form kvas beverage comprising the step of mixing either i) concentrated fermented kvas wort with sweetener, water and optionally food acids and an aromatic solution comprising at least 20% v/v ethanol or ii) a fermentate of a wort comprising 4-13% v/v ethanol with sweetener, water and optionally unfermented kvas wort concentrate and/or food acids.
9. The method of claim 8 wherein the concentrated fermented kvas wort is obtained by a method of any one of claims 1 steps a) to e) or claims 2 to 7.
10. The method of claim 8 wherein the fermentate is obtained by a method of any one of claims 1 steps ai) to ci) or claims 2 to 5.
11. Concentrated fermented kvas wort obtainable by a method of any one of claims 1 steps a) to e) or 2 to 7.
12. Fermentate obtainable by a method of any one of claims 1 steps ai) to ci) or 2 to 5.
13. A syrup obtainable by the method of any one of claims 8 to 10.
14. Kvas beverage obtainable by the method of any one of claims 8 to 10 or by diluting the syrup of claim 13 with still or carbonated water.

Figure 1

Current technology of kvas manufacturing



Food acids are used only in case of fermentation solely with yeast. In case of combined fermentation with lactobacteria and yeast addition of acids is not needed.

Figure 2

New process of kvas production

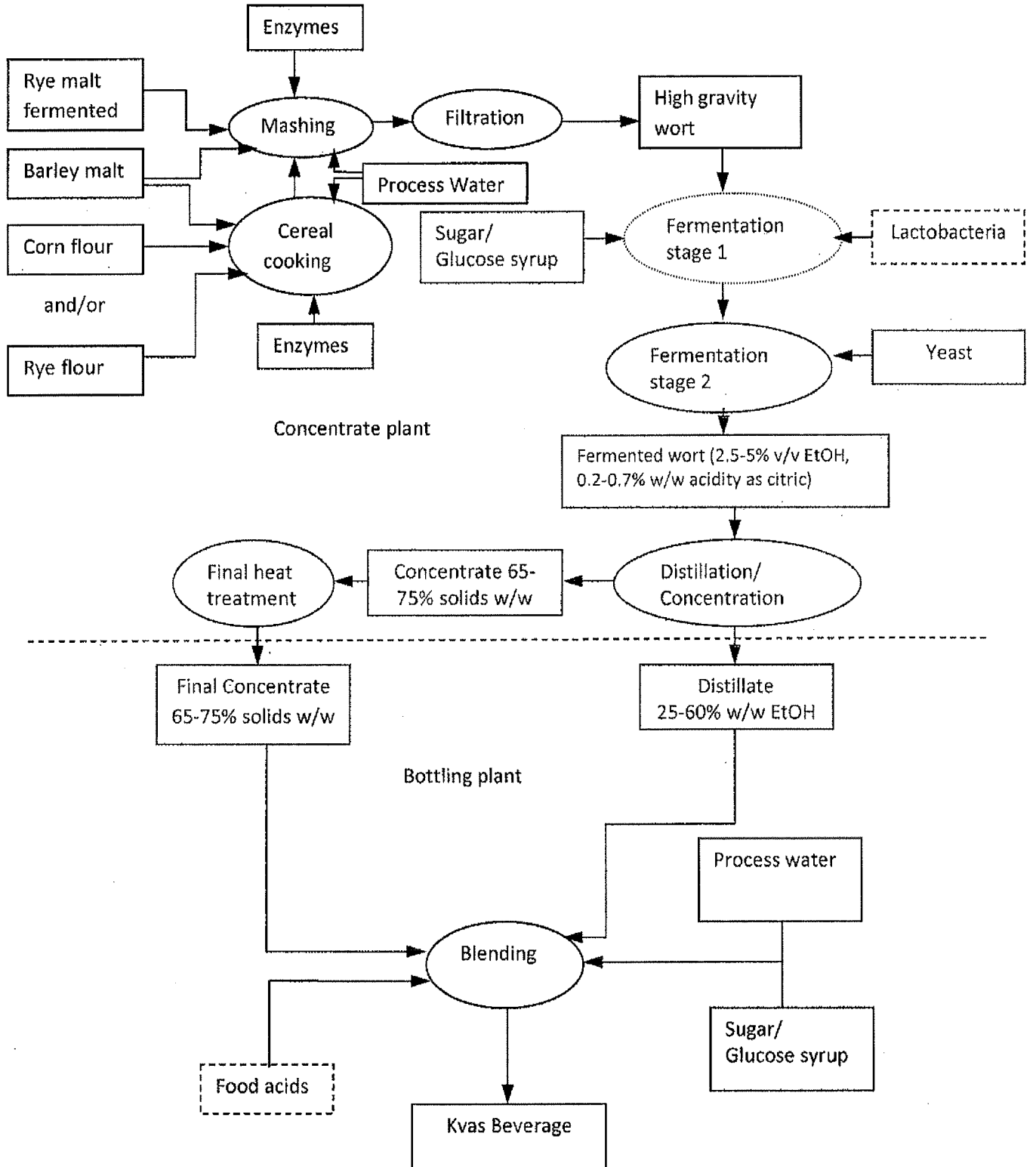
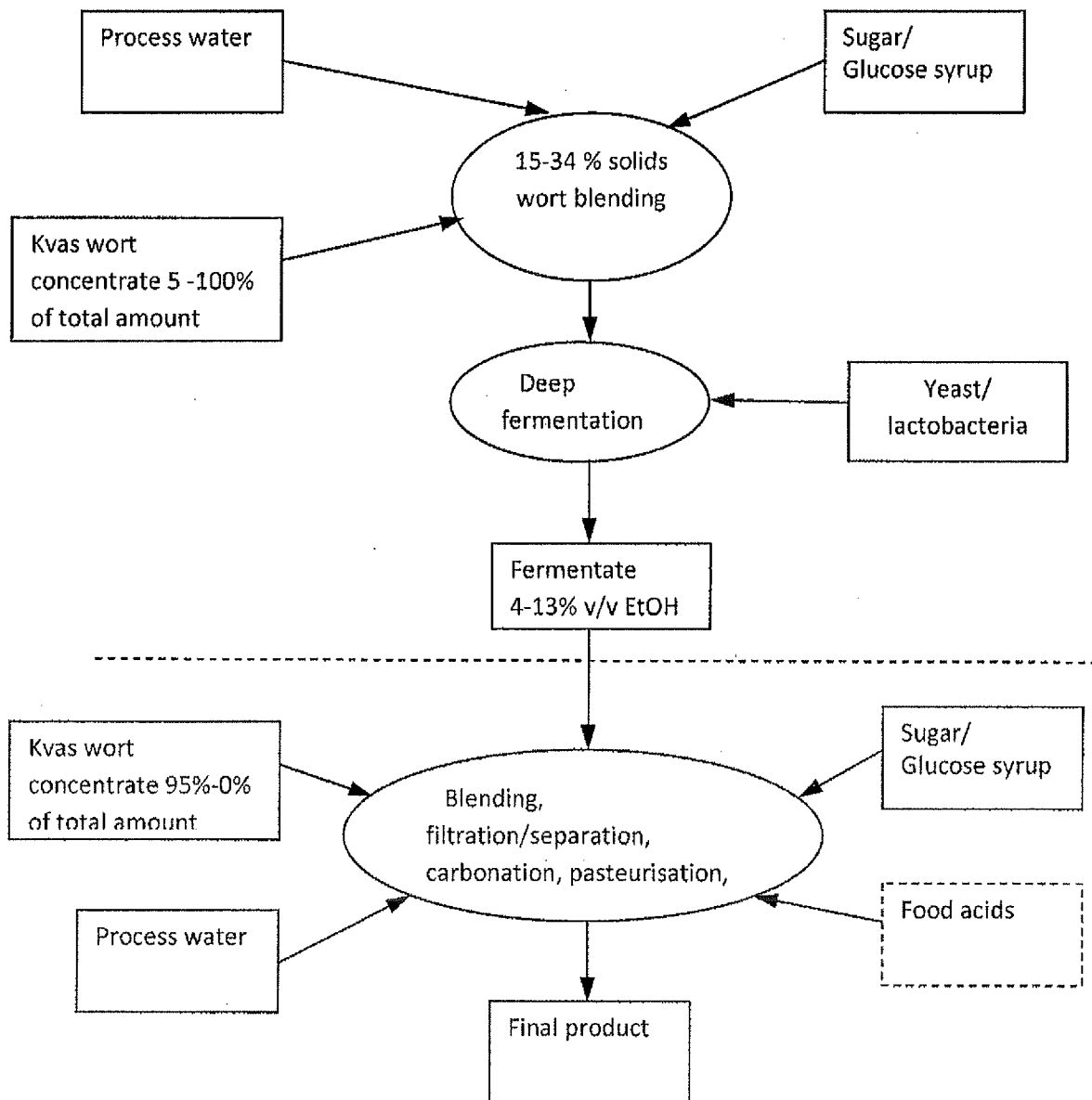


Figure 3

New process of kvas production



Blending process

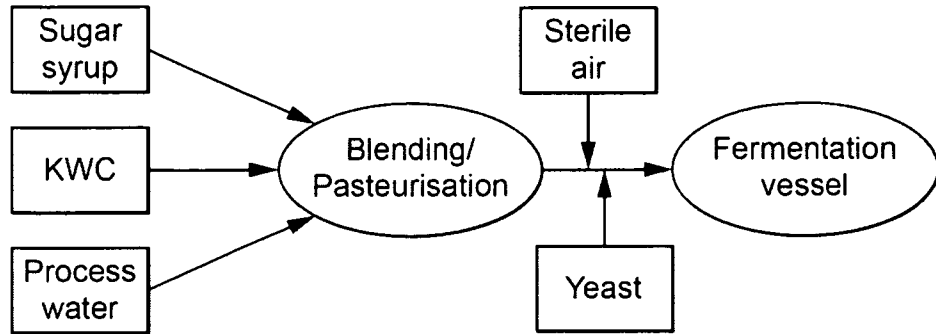


FIG. 4.1

Fermentation diagram

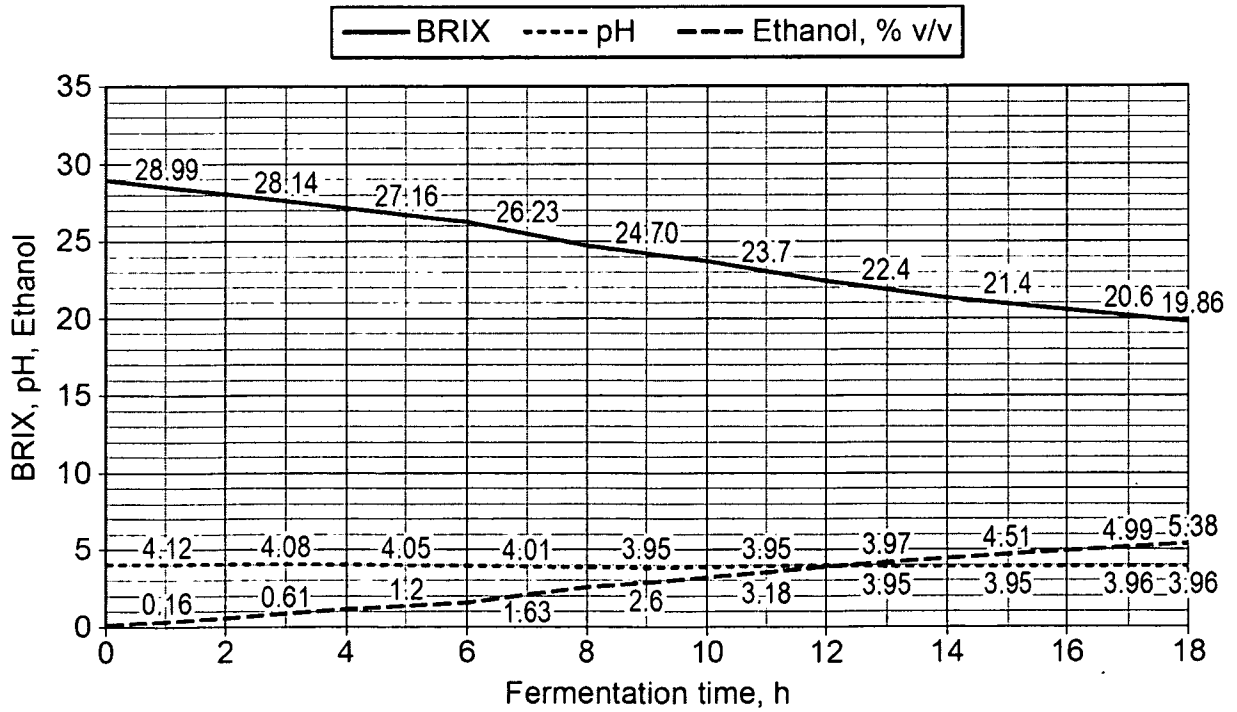


FIG. 4.2

Figure 4.3

Filling process

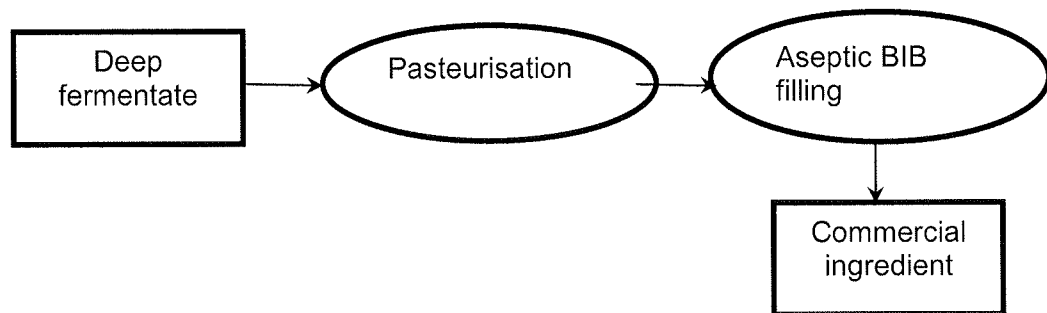
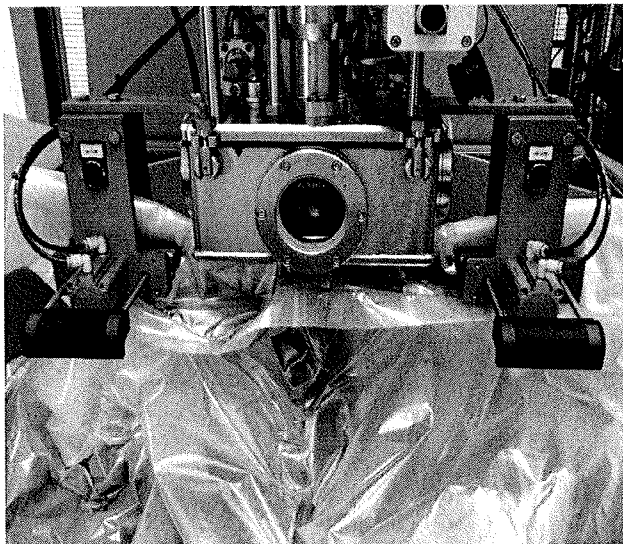


Figure 4.4

Aseptic filling of BIB



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/068279

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12C7/00 C12C12/00 C12G3/00
 ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 C12C C12G A23L

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 EPO-Internal, WPI Data, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	column 2, line 64 - column 5, line 2 & DATABASE WPI Week 198419 Thomson Scientific, London, GB; AN 1984-119000 XP002686884, & SU 1 033 116 A (NOVIKOV E P) 7 August 1983 (1983-08-07) abstract	3,4
X	----- SU 1 409 650 A1 (BOJKO NIKOLAJ P [SU]) 15 July 1988 (1988-07-15)	1,2,5,6, 8-14
Y	the whole document -/--	3,4

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search 12 November 2012	Date of mailing of the international search report 22/11/2012
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Krajewski, Doris

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/068279

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y A	& DATABASE WPI Week 198944 Thomson Scientific, London, GB; AN 1989-322063 XP002686886, & SU 1 450 814 A (MINERAL WATER IND) 15 January 1989 (1989-01-15) abstract	3,4 1,2,5-10
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/068279

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RU 2333947	C2	20-09-2008	NONE
