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(54) Title: METHOD FOR PRODUCTION OF BREWERS WORT

(57) Abstract: A method of producing a brewer's wort without using malted grains comprising: a) Providing a mash from a grist comprising sorghum; b) Adding an alpha-amylase and a glucoamylase to the mash; c) Performing an infusion mashing; d) Obtaining a wort with more than 75% (w/w) glucose compared to the initial total carbohydrate of the grist, and wherein the glucoamylase has at least 70% identity to the sequence shown in SEQ ID NO: 1.



METHOD FOR PRODUCTION OF BREWERS WORT

REFERENCE TO A SEQUENCE LISTING

This application contains a Sequence Listing in computer readable form. The computer
5 readable form is incorporated herein by reference.

FIELD OF INVENTION

The present invention relates to a method for production of a brewer's wort using sorghum
and not any malted grains as the grist.

BACKGROUND OF THE INVENTION

In modern mashing processes, enzymes are often added as a supplement when mashing
malt and adjunct grist. Enzymes may also be applied in mashing of well modified malts with high
enzyme content to increase the extract recovery and the amount of fermentable sugars, as well as
15 accelerate the overall conversion time.

Matthews et al., 2001, Journal of Institute of brewing 107(3) pp185-194, discloses preparation
of a low carbohydrate beer by mashing a malt grist with a glucoamylase, which is derived from
Aspergillus niger.

WO 2012/140075 describes the use of a glucoamylase from *Penicillium oxalicum* when
20 mashing a malt and adjunct grist.

When working with a grist of sorghum instead of barley, current solutions normally involve a
long decoction mashing program.

Therefore, there stills exists a need for methods that would help reduce mashing time and
maximize the glucose level in the production of brewer's wort when using 100% un-malted grains
25 comprising sorghum.

SUMMARY OF THE INVENTION

The present inventors have surprisingly found that it is possible, when using un-malted
sorghum as the grist, to have short mashing times and to obtain a high glucose concentration in the
30 wort, so we claim:

A method of producing a brewer's wort without using malted grains comprising

- a) Providing a mash from a grist comprising sorghum;
- b) Adding an alpha-amylase and a glucoamylase to the mash;

c) Performing an infusion mashing;

d) Obtaining a wort with more than 75% (w/w) glucose compared to the initial total carbohydrate of the grist, and

wherein the glucoamylase has at least 70% identity to the sequence shown in SEQ ID NO: 1.

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In one embodiment, the sorghum is white sorghum.

In one embodiment, the alpha-amylase has at least at least 70% identity to the sequence shown in SEQ ID NO: 2.

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In one embodiment, the mashing comprises an incubation step of at least 75°C for at least 30 minutes.

In one embodiment, the wort has more than 76 % (w/w) glucose, or more than 77 % (w/w) glucose; or more than 78 % (w/w) glucose; or more than 79 % (w/w) glucose; or more than 80 % (w/w) glucose; or more than 81 % (w/w) glucose; or more than 82 % (w/w) glucose; or more than 83 % (w/w) glucose; or more than 84 % (w/w) glucose; or more than 85 % (w/w) glucose; or more than 86 % (w/w) glucose; or more than 87 % (w/w) glucose; or more than 88 % (w/w) glucose; or more than 89 % (w/w) glucose; or more than 90 % (w/w) glucose; or more than 91 % (w/w) glucose; or more than 92 % (w/w) glucose; or more than 93 % (w/w) glucose; or more than 94 % (w/w) glucose; or more than 95 % (w/w) glucose; compared to the initial total carbohydrate of the grist.

20

In one embodiment, the method further comprises adding a protease.

In one embodiment, the method further comprises adding a xylanase.

25

In one embodiment, the method further comprises adding a lipase.

In one embodiment, the method further comprises adding a cellulase.

In one embodiment, the alpha-amylase is added in an amount of 0.5 to 500 mg enzyme protein per kg grist.

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In one embodiment, the glucoamylase is added in an amount of 1 to 1000 mg enzyme protein per kg grist.

In one embodiment, the grist comprises at least 40% (w/w) sorghum.

In one embodiment, the total mashing time is less than 130 minutes.

In one embodiment, the wort is converted to beer.

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DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term "grist" is understood as the starch or sugar containing material that is the basis for beer production.

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The term "malt" is understood as any malted cereal grain.

The term "adjunct" is understood as the part of the grist which is not malt.

The term "mash" is understood as a starch containing slurry of the grist comprising crushed grain, optionally other starch containing material, or a combination thereof, steeped in water to make wort.

15

The term "mashing" is the process of converting starch in the mash into fermentable and un-fermentable sugars.

The term "wort" is understood as the unfermented liquor run-off, following extracting the grist during mashing.

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The term "RDF" (real degree of fermentation) measures the degree to which sugar in wort may be fermented into alcohol in beer, defined as "attenuation."

The terms "DP1" (Degree of polymerization 1) denotes glucose or fructose, "DP2" denotes maltose or isomaltose, and DP3 denotes maltotriose. The terms "DP4+" or "DP4/4+" denote dextrin, or maltooligosaccharides of a polymerization degree of 4 or higher.

25

The term "identity" is the relatedness between two amino acid sequences. For purposes of the present invention, the degree of identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, Trends in Genetics 16: 276-277), preferably version 5.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows:

30

$$(\text{Identical Residues} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$$

Method of producing a brewer's wort and a beer according to the invention

Conventional machinery, equipment, and materials can be used during mashing.

5 The grist is mixed with water prior to mashing. The water may, before being added to the grist, be preheated in order for the mash to attain the desired mash temperature at the moment of mash forming.

If the temperature of the formed mash is below the desired mashing temperature, additional heat is preferably supplied in order to attain the desired start process temperature.

10 Preferably, the desired start mashing temperature is attained within 15 minutes, or more preferably within 10 minutes, such as within 9, 8, 7, 6, 5, 4, 3, 2 minutes or even more preferably within 1 minute after the mash forming, or most preferably the desired mashing temperature is attained at the mash forming.

According to the present invention, the mash contains no malted grains.

15 According to the present invention, the grist comprises un-malted sorghum; e.g., the grist comprises 100% un-malted sorghum (w/w); e.g., the grist comprises at least 95% (w/w) un-malted sorghum; e.g., the grist comprises at least 90% (w/w) un-malted sorghum; e.g., the grist comprises at least 85%(w/w) un-malted sorghum; e.g., the grist comprises at least 80% (w/w) un-malted sorghum; e.g., the grist comprises at least 75% (w/w) un-malted sorghum; e.g., the grist comprises at least 70% (w/w) un-malted sorghum; e.g., the grist comprises at least 60% (w/w) un-malted
20 sorghum; e.g., the grist comprises at least 55% (w/w) un-malted sorghum; e.g., the grist comprises at least 50% (w/w) un-malted sorghum; e.g., the grist comprises at least 45% (w/w) un-malted sorghum; e.g., the grist comprises 40% (w/w) un-malted sorghum.

25 According to the present invention, any sorghum may be used, in particular white sorghum or red sorghum. Red sorghum is red due to polyphenolic pigments. White sorghum does not contain polyphenolic pigments.

According to the present invention, the sorghum may be chosen from one or more of the following species:

1. Sorghum amplum
2. Sorghum angustum
- 30 3. Sorghum arundinaceum
4. Sorghum bicolor
5. Sorghum brachypodium
6. Sorghum bulbosum

7. *Sorghum burmahicum*
8. *Sorghum controversum*
9. *Sorghum drummondii*
10. *Sorghum ecarinatum*
- 5 11. *Sorghum exstans*
12. *Sorghum grande*
13. *Sorghum halepense*
14. *Sorghum interjectum*
15. *Sorghum intrans*
- 10 16. *Sorghum laxiflorum*
17. *Sorghum leiocladum*
18. *Sorghum macrospermum*
19. *Sorghum matarankense*
20. *Sorghum nitidum*
- 15 21. *Sorghum plumosum*
22. *Sorghum propinquum*
23. *Sorghum purpureosericeum*
24. *Sorghum stipoides*
25. *Sorghum timorense*
- 20 26. *Sorghum trichocladum*
27. *Sorghum versicolor*
28. *Sorghum virgatum*

According to the present invention, a preferred sorghum is the species *Sorghum bicolor*.

According to the present invention, a preferred sorghum is white sorghum.

25 In addition to the sorghum, the grist may comprise other un-malted grains such as barley, wheat, rye, oat, maize, rice, and/or millet, or raw and/or refined starch and/or sugar containing material derived from plants like wheat, rye, oat, maize, rice, milo, millet, sorghum, potato, sweet potato, cassava, tapioca, sago, banana, sugar beet and/or sugar cane.

30 The adjuncts may be obtained from tubers, roots, stems, leaves, legumes, cereals and/or whole grain.

In a preferred embodiment, the grist comprises sorghum and cassava.

Preferably, these other adjuncts have high gelatinization temperature. More particularly, these adjuncts have a high onset gelatinization temperature.

Adjunct may also comprise readily fermentable carbohydrates such as sugars or syrups and they may be added to the mash before, during or after the mashing process of the invention, but is preferably added after the mashing process. According to the present invention, the amount of added sugars or syrups may be reduced by using the method of the invention.

Prior to forming the mash, the adjunct is preferably milled and most preferably dry or wet milled.

The enzymes may be added as enzyme compositions. They may consist of one enzyme or more than one enzyme. The enzyme composition, in addition to the enzyme(s), may also contain at least one other substance, for example but not limited to buffer, surfactants, etc. The enzyme compositions may be in any form, for example, solid, liquid, emulsion, gel, or paste. Such forms are known to the person skilled in the art.

In one aspect of the invention, an enzyme composition comprising a glucoamylase and an alpha amylase may be added to the mash ingredients, e.g., the water or the grist before, during or after forming the mash, or at any time during the mashing.

During the mashing process according to the invention, starch extracted from the grist is gradually hydrolysed into fermentable sugars, smaller dextrins, and glucose.

The mashing may comprise an incubation step of at least 75°C for at least 30 minutes, e.g., an incubation step of at least 76°C for at least 30 minutes; e.g., an incubation step of at least 77°C for at least 30 minutes; e.g., an incubation step of at least 78°C for at least 30 minutes; e.g., an incubation step of at least 79°C for at least 30 minutes, e.g., an incubation step of at least 80°C for at least 30 minutes.

After mashing, when all the starch has been broken down, it is necessary to separate the liquid extract (the wort) from the solids (spent grains).

Wort separation, lautering, is important because the solids contain large amounts of protein, poorly modified starch, fatty material, silicates, and polyphenols (tannins).

According to the present invention, a mashing-off step may not be necessary because the incubation may be performed at a high temperature, typically 75-80°C.

Following the separation of the wort from the spent grains, the wort may be fermented with brewer's yeast to produce a beer.

The extract retained in the spent grain after collection of the first wort may also be washed out by adding hot water on top of the lautер cake. This process is called sparging. The hot water flows through the spent grain and dissolves the remaining extract. The diluted wort is called second wort.

After optional addition of hops, the wort is boiled. Hereby, numerous substances including several proteins are denatured, and a precipitation of polyphenols will take place.

After cooling and removal of the precipitates, the finished beer wort is aerated, and yeast is added.

5 The yeast applied may be any yeast suitable for beer brewing, especially yeasts selected from *Saccharomyces* such as *S. cerevisiae* and *S. uvarum*, including natural or artificially produced variants of these organisms.

10 After a main fermentation, lasting typically 5-10 days, most of the yeast is removed, and the so-called green beer is stored at a low temperature, typically at 0°C - 5°C for 1 to 12 weeks. During this period the remaining yeast will precipitate together with polyphenols. To remove the remaining excess polyphenols, a filtration may be performed.

The fermented beer may now be carbonized prior to bottling. Carbon dioxide not only contributes to the perceived "fullness" or "body" and as a flavor enhancer, it also acts as an enhancer of the foaming potential and plays an important role in extending the shelf life of the product.

15 Further information on conventional brewing processes may be found in "Technology Brewing and Malting" by Wolfgang Kunze of the Research and Teaching Institute of Brewing, Berlin (VLB), 2nd revised Edition 1999, ISBN 3-921690-39-0.

20 Any beer type may be produced from the wort according to the present invention. Preferred beer types comprise ales, strong ales, stouts, porters, lagers, bitters, export beers, malt liquors, happoushu, high-alcohol beer, low-alcohol beer, low-calorie beer, or light beer.

The wort may also be processed to be used as syrups. It may also be used to produce non-alcoholic beverages. These processes are well known to a person skilled in the art.

25 According to the present invention, the wort has more than 75 % (w/w) glucose; or more than 76 % (w/w) glucose; or more than 77 % (w/w) glucose; or more than 78 % (w/w) glucose; or more than 79 % (w/w) glucose; or more than 80 % (w/w) glucose; or more than 81 % (w/w) glucose; or more than 82 % (w/w) glucose; or more than 83 % (w/w) glucose; or more than 84 % (w/w) glucose; or more than 85 % (w/w) glucose; or more than 86 % (w/w) glucose; or more than 87 % (w/w) glucose; or more than 88 % (w/w) glucose; or more than 89 % (w/w) glucose; or more than 90 % (w/w) glucose; or more than 91 % (w/w) glucose; or more than 92 % (w/w) glucose; or more than 93 % (w/w) glucose; or more than 94 % (w/w) glucose; or more than 95 % (w/w) glucose; compared to the initial
30 total carbohydrate of the grist.

In one aspect, the method of the invention leads to shortened mashing times. In one aspect, the method leads to decrease in mashing time by at least 5 minutes e.g., at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes more preferably by at least 30 minutes compared

to the method done in the absence of the glucoamylase according to the invention.

In one aspect, the method of the invention leads to mashing times that are below 130 minutes. In another aspect, the mashing time is below 120 minutes, e.g. below 110 minutes, e.g. below 100 minutes, e.g., below 90 minutes, e.g., below 80 minutes, e.g., below 70 minutes, e.g., below 60 minutes, e.g., below 50 minutes, e.g., below 40 minutes.

ENZYMES

Glucoamylase (EC 3.2.1.3)

Glucoamylases (Glucan 1,4- α -glucosidase) are enzymes which catalyze the hydrolysis of terminal (1-4)-linked α -D-glucose residues successively from non-reducing ends of the chains with release of β -D-glucose.

According to the present invention, the glucoamylase has at least 70% identity to the sequence shown in SEQ ID NO: 1.

In one aspect, the glucoamylase has an amino acid sequence which is at least 71%, at least 72 %, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or even at least 99% identical to the amino acid sequence shown in SEQ ID NO: 1.

In one embodiment, the glucoamylase according to the present invention is SEQ ID NO: 1.

In a preferred embodiment, the glucoamylase has an amino acid sequence which differs by no more than 100 amino acids, preferably by no more than 80 amino acids, more preferred by no more than 50 amino acids, more preferably by no more than 30 amino acids, even more preferably by no more than 20 amino acids, and most preferably by no more than 10 amino acids from the amino acid sequence of SEQ ID NO: 1.

In one aspect, the glucoamylase is added in a concentration of 1 to 1000 mg of enzyme protein (EP) per kg of total weight of the grist, e.g., 10 to 1000 mg of enzyme protein (EP) per kg of total weight of the grist, e.g., 50 to 500 mg of enzyme protein (EP) per kg of total weight of the grist.

Alpha-amylase (EC 3.2.1.1)

An alpha-amylase according to the invention may be only microbial alpha-amylase. The alpha-amylase may be a *Bacillus* alpha-amylase. Well-known *Bacillus* alpha-amylases include alpha-amylase derived from a strain of *B. licheniformis*, *B. amyloliquefaciens*, and *B. stearothermophilus*.

A preferable alpha amylase is SEQ ID NO:2.

In one aspect of the invention, the alpha amylase has at least 70% identity, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% or even 100% identity to the sequence shown in SEQ ID NO:2.

In one aspect, the alpha amylase is added in a concentration of 0.5 to 500 mg of enzyme protein (EP) per kg of total weight of the grist, e.g., 5 to 500 mg of enzyme protein (EP) per kg of total weight of the grist, e.g., 10 to 100 mg of enzyme protein (EP) per kg of total weight of the grist.

Other enzymes

In another preferred embodiment, a further enzyme(s) is added to the mash, said enzyme(s) including but not limited to isoamylase, protease, cellulase, glucanase, laccase, xylanase, lipase, phospholipolase, phytase, phytin, and esterase.

In one aspect of the method, the further enzyme added includes but is not limited to a protease.

In one aspect of the method, the further enzyme added includes but is not limited to a cellulase.

In one aspect of the method, the further enzyme added includes but is not limited to a xylanase. In an especially preferred embodiment, the xylanase is a GH30 xylanase.

In one aspect of the method, the further enzyme added includes but is not limited to a lipase.

EXAMPLES

Example 1

100% un-malted Sorghum infusion mashing

The mashing was carried out at two different dosages, all done in quadruplicates in a Lochner mashing bath.

Each beaker in the mashing bath contained 50 g disc-milled (0.2 mm gap) white sorghum from Kenya and 150 ml DI-water containing approximately 100 ppm Ca^{2+} .

The pH was adjusted to 5.4 – 5.5 at the mash-in temperature, and the enzymes were added afterwards. The following enzyme blends were added:

Blend 1: 240 mg enzyme protein of SEQ ID NO:1: and 27 mg enzyme protein of SEQ ID NO:2 per kg grist

- 5 Blend 2: 300 mg enzyme protein of SEQ ID NO:1: and 33 mg enzyme protein of SEQ ID NO:2 per kg grist

The mashing program was started immediately afterwards: 65°C for 0 min, heating to 80°C by 1°C/min, 60 min rest at 80°C, so the total mashing time was 75 min.

- 10 The mashing program was terminated by cooling the beakers to 20°C before adjusting the mass in each beaker to 250 g before filtering the liquid (wort) from the solids (spent grains) through a paper filter in a funnel.

Mash-in: 65°C Time: 0 min

Heating: 1°C/min

- 15 Mash-off: 80°C Time: 60 min

The resulting wort was analyzed for sugar profile by HPLC. Results are shown in Table 1:

Table 1:

Sugar profile (in %) of the wort after mashing and filtration. (DP = Degree of Polymerization)

Enzymes	%DP4+	%DP3	%DP2	%DP1
Blend 1	9.6	1.3	8.1	81.1
Blend 2	7.5	1.4	5.4	85.6

20

It can be seen from Table 1 that it is possible to obtain a very high DP1 – higher than 80% - when using the method according to the invention.

Example 2

- 25 Mashing and fermentation of 100% un-malted Sorghum

The mashing was carried out in a Lochner mashing bath using six beakers, each containing 50 g disc-milled (0.2 mm gap) un-malted white sorghum and 175 ml DI-water containing approximately 100 ppm Ca^{2+} .

- 5 The pH was adjusted to 5.4 – 5.5 at the mash-in temperature. The enzymes were then added.

Blend 1: 300 mg enzyme protein of SEQ ID NO:1: and 33 mg enzyme protein of SEQ ID NO:2 per kg grist

The mashing program was started immediately after dosing the enzymes: 65°C for 0 min, heating to 80°C by 1°C/min, 60 min rest at 80°C.

- 10 The mashing was terminated by cooling to 20°C, then the mass in the beakers was adjusted to 250g with DI-water, before filtering the mash in the beakers through a paper filter in a funnel.

The resulting wort from beaker 1 and 2 was mixed together, as was the wort from beaker 3 and 4, and beaker 5 and 6.

- 15 200 ml from each of the mixes was boiled for 40 minutes with bittering hops to achieve an IBU of 15, cooled to 20°C, adjusted to pH 5.3.

175 ml of each was transferred to 250 ml blue cap bottles, pasteurized, and pitched with freshly propagated yeast (Saflager W-34/70) to obtain 12 million cells/ml.

The blue cap bottles were placed at 12°C on a magnetic stirrer for two weeks.

- 20 Samples were taken during the fermentation and of the resulting beer. The samples were analyzed, and the results are shown in Table 2 and 3:

Table 2:

Wort data

	Replicate 1	Replicate 2	Replicate 3
Extract (°P)	14.58	14.56	14.55
DP1 (%)	78.6	78.6	78.5
DP2 (%)	10.0	10.0	10.0
DP3 (%)	0.8	0.8	0.8
DP4/DP4+ (%)	10.6	10.6	10.6

Table 3:

Fermentation data

Days of fermentation		0	3	4	7	10	12	13	14	ABV (%)	RDF (%)
Replicate 1	DP1 (g/l)	107.5	43.6	27.4	0.7	0.7	0.7	0.7	0.7	7.4	81.6
	DP2 (g/l)	10.4	10.0	9.4	9.4	4.2	2.1	2.0	1.9		
	DP3 (g/l)	1.4	1.4	1.4	1.4	1.3	1.3	1.2	1.1		
	DP4/DP4+ (g/l)	14.4	14.4	13.8	14.6	14.5	14.8	14.6	14.1		
	Total sugars (g/l)	133.7	69.4	52.0	26.1	20.7	18.9	18.5	17.9		
Replicate 2	DP1 (g/l)	108.6	40.5	24.9	1.1	1.5	1.5	1.8	1.4	7.5	81.4
	DP2 (g/l)	10.6	9.6	9.2	9.9	7.9	5.1	3.4	2.3		
	DP3 (g/l)	1.4	1.4	1.3	1.3	1.3	1.3	1.3	1.2		
	DP4/DP4+ (g/l)	14.7	14.1	13.7	14.6	14.6	14.6	14.7	14.4		
	Total sugars (g/l)	135.3	65.5	49.2	27.0	25.4	22.5	21.2	19.2		
Replicate 3	DP1 (g/l)	108.8	43.4	29.2	1.1	1.3	0.7	0.8	0.9	7.4	80.1
	DP2 (g/l)	10.6	10.3	10.0	10.7	10.2	9.2	8.4	7.1		
	DP3 (g/l)	1.3	1.4	1.4	1.4	1.3	1.3	1.3	1.3		
	DP4/DP4+ (g/l)	14.6	14.7	14.6	14.8	14.9	15.0	14.8	14.4		
	Total sugars (g/l)	135.3	69.8	55.1	27.9	27.7	26.3	25.3	23.7		

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Table 2 and 3 show that it was possible to mash the 100% white sorghum with the two enzymes, obtaining a high DP1 and resulting in a Real Degree of Fermentation of >80%.

Example 3

10 Brewing a 100% un-malted sorghum beer

The raw material was 100% un-malted white sorghum.

The white sorghum was milled using hammermilling.

The pH was adjusted to 5.4 at the mash-in temperature. The enzymes were added at mash-in, after 1/3 of grist had been added.

15 The following enzyme blend was added:

Blend 1: 180 mg enzyme protein of SEQ ID NO:1 and 20 mg enzyme protein of SEQ ID NO:2 per kg grist

It may not be necessary to add a protease, but in order to secure that a sufficient level of amino acids was present during fermentation, a protease was added (2.0 g/kg grist of FAN Boost™ (Novozymes A/S)).

The mashing regime was 65°C/20min - 80°C/60min.

The fermentation conditions used were a temperature of 20°C with a top-fermenting yeast.

Table 4:

The following wort and beer results were achieved:

Day of fermentation	0	1	2	3	4	5	6	7	8	13
Apparent extract (*Plato)	16.11	15.78	11.93	9.13	7.07	5.30	3.64	2.14	0.90	0.40
Alcohol (%V/V)	0.00	0.04	2.11	3.62	4.72	5.67	6.56	7.30	7.03	7.10
RDF (%)	0.0	0.4	20.6	35.4	46.2	55.4	64.0	71.5	77.1	79.8
DP1 (g/l)	138.7	130.8	93.7	72.0	55.0	40.7	25.9	14.8	4.2	0.6
DP2 (g/l)	12.6	12.2	11.1	10.7	10.3	10.1	9.5	9.3	7.7	6.7
DP3 (g/l)	2.6	2.5	2.4	2.5	2.5	2.5	2.5	2.6	2.2	2.1
DP4/DP4+ (g/l)	13.1	12.9	12.6	13.1	13.1	13.6	13.1	13.5	11.7	11.3
Total sugars (g/l)	167.0	158.4	119.9	98.3	80.9	66.9	51.0	40.2	25.9	20.8
DP1 (%)	82									
DP2 (%)	8									
DP3 (%)	1									
DP4/DP4+ (%)	9									

Comments:

The brewed beers were good; and RDF, alcohol percentage, and sugar profile were excellent.

Example 4

50% un-malted sorghum and 50% cassava flour infusion mashing

The mashing was carried out at three different dosages, all done in quadruplicates in a Lochner mashing bath.

- 5 Each beaker in the mashing bath contained 25 g disc-milled (0.2 mm gap) white sorghum from Uganda, 25 g cassava flour from Uganda, and 150 ml DI-water containing approximately 100 ppm Ca^{2+} .

The pH was adjusted to 5.4 – 5.5 at the mash-in temperature, and the enzymes were added afterwards. The following enzyme blends were added:

- 10 Blend 1: 180 mg enzyme protein of SEQ ID NO:1: and 21 mg enzyme protein of SEQ ID NO:2 per kg grist
- Blend 2: 240 mg enzyme protein of SEQ ID NO:1: and 27 mg enzyme protein of SEQ ID NO:2 per kg grist
- 15 Blend 3: 300 mg enzyme protein of SEQ ID NO:1: and 33 mg enzyme protein of SEQ ID NO:2 per kg grist

The mashing program was started immediately afterwards: 40°C for 5 min, heating to 80°C by 1°C/min, 60 min rest at 80°C, resulting in a total mashing time of 105 min.

The mashing program was terminated by cooling the beakers to 20°C.

- 20 The mass in each beaker was then adjusted to 250 g before filtering the liquid (wort) from the solids (spent grains) through a paper filter in a funnel.

Mash-in: 40°C Time: 5 min

Heating: 1°C/min

Mash-off: 80°C Time: 60 min

- 25 The resulting wort was analyzed for sugar profile by HPLC. The results are shown in Table 5:

Table 5:

Sugar profile (in %) of the wort after mashing and filtration. (DP = Degree of Polymerization)

Enzymes	%DP4+	%DP3	%DP2	%DP1
Blend 1	8.5	1.7	5.4	84.5
Blend 2	5.4	1.7	2.6	90.2
Blend 3	3.6	1.5	1.9	92.9

It can be seen from Table 5 that it is possible to obtain a very high DP1 – higher than 90% - when
5 using the method according to the invention.

CLAIMS

1. A method of producing a brewer's wort without using malted grains comprising
 - a) Providing a mash from a grist comprising sorghum;
 - b) Adding an alpha-amylase and a glucoamylase to the mash;
 - c) Performing an infusion mashing;
 - d) Obtaining a wort with more than 75% (w/w) glucose compared to the initial total carbohydrate of the grist, andwherein the glucoamylase has at least 70% identity to the sequence shown in SEQ ID NO: 1.
2. The method according to claim 1, wherein the sorghum is white sorghum.
3. The method according to any of the preceding claims, wherein the alpha-amylase has at least at least 70% identity to the sequence shown in SEQ ID NO: 2.
4. The method according to any of the preceding claims, wherein the mashing comprises an incubation step of at least 75°C for at least 30 minutes.
5. The method according to any of the preceding claims, wherein the wort has more than 76 % (w/w) glucose, or more than 77 % (w/w) glucose; or more than 78 % (w/w) glucose; or more than 79 % (w/w) glucose; or more than 80 % (w/w) glucose; or more than 81 % (w/w) glucose; or more than 82 % (w/w) glucose; or more than 83 % (w/w) glucose; or more than 84 % (w/w) glucose; or more than 85 % (w/w) glucose; or more than 86 % (w/w) glucose; or more than 87 % (w/w) glucose; or more than 88 % (w/w) glucose; or more than 89 % (w/w) glucose; or more than 90 % (w/w) glucose; or more than 91 % (w/w) glucose; or more than 92 % (w/w) glucose; or more than 93 % (w/w) glucose; or more than 94 % (w/w) glucose; or more than 95 % (w/w) glucose; compared to the initial total carbohydrate of the grist.
6. The method according to any of the preceding claims, further comprising adding a protease.
7. The method according to any of the preceding claims, further comprising adding a xylanase.

8. The method according to any of the preceding claims, further comprising adding a lipase.
9. The method according to any of the preceding claims, further comprising adding a cellulase.
- 5 10. The method according to any of the preceding claims, wherein the alpha-amylase is added in an amount of 0.5 to 500 mg enzyme protein per kg grist.
11. The method according to any of the preceding claims, wherein the glucoamylase is added in an amount of 1 to 1000 mg enzyme protein per kg grist.
- 10 12. The method according to any of the preceding claims, wherein the grist comprises at least 40% (w/w) un-malted sorghum.
13. The method according to any of the preceding claims, wherein the total mashing time is less than 130 minutes.
- 15 14. The method according to any of the preceding claims, wherein the wort is converted to beer.