

PATENT SPECIFICATION (11)

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(54) PROCESS FOR THE PRODUCTION OF BEER WORT

(71) We, CIBA-GEIGY AG, a Swiss Body Corporate of Basle, Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

The present invention relates to a process for the production of beer wort using unmalted grain, with the aid of added enzymes and to beer wort produced thereby.

Beer is produced by the fermentation of beer wort which in turn is obtained from malt. The production of malt, in turn, is based on the germination (malting) and subsequent drying of brewer's barley at an elevated temperature (kiln-drying) in installations especially equipped for this purpose (malt houses). It is possible to use only selected varieties of barley as brewer's barley because certain quality aspects such as, *inter alia*, good germinating power and a high overall standard of quality are required.

In order to meet these requirements, substantial agronomic and technological effort is required, in the form of knowledge, equipment and investment. Malt is therefore a relatively expensive starting material for the production of beer.

Hence there is a need in the brewing industry to replace malt wholly or at least largely by so-called unmalted grain which contains, *inter alia*, starch and protein. Normally, the enzymes originally formed in the malt digest the material introduced in the mash, this digestion being necessary for the process of brewing. If, however, the malt is entirely or largely absent when making the mash, and unmalted grain is employed instead, there is a shortage of the requisite enzyme activities for digesting the unmalted grain in the way required for the brewing process. This shortage can be rectified by addition of suitable enzymes.

Processes have already been proposed which employ enzymes of animal, vegetables or microbial origin. The enzymes mainly employed are amylases and proteases and also amyloglucosidases and β -glucanases.

Examples of the use of enzymes for this purpose are known from the literature, including, *inter alia*, Allgem. Br. Hopf. Ztg. 73 (1933) 700; W.F.Br. 56 (1939) 161; Stärke 3 (1951) 155-159; Brauwelt 37 (1952) 863; Petit J. Brasseur 65 (1957); Fermentatio (1958) 99-126; British Patent Specification No. 513,455 (1939); Canadian Patent Specification No. 634,865 (1962); and Belgian Patent Specifications Nos. 753,174 (1970) and 754,132 (1970).

These proposals, however, provide an inadequate solution to the problem indicated above inasmuch as the enzyme additives recommended in the literature do not make it possible to match the enzymatic activities to the particular unmalted grain additives in the way required in practice. The reason for this is, first, that these enzyme preparations contain several enzyme activities of different specificity of action, so that it is not possible to control the enzymatisation steps of the brewing process in a way which suits the substrate. Secondly, the known processes of production of beer, based on the use of these enzyme preparations cannot be satisfactorily reproduced since the enzyme preparations do not possess a constant and perfectly reproducible specificity of action.

Instead, such enzyme preparations show variable and frequently inadequate activities because of the difficulties of controlling the individual enzyme activities. Furthermore, it has been found that preparations which contain enzymes of different activities, for example protease and amylases, are not well suited to controlling use in the production of beer, because the different individual activities cannot be controlled separately and hence cannot be matched perfectly to the circumstances of the particular brewing process, which depends, *inter alia*, on the nature and amount of unmalted grain added. It is not possible, therefore, to obtain, using the prior enzyme preparations, optimum equilibrium between saccharification and proteolysis, which is a decisive

factor in the desired course of the brewing process. The above disadvantages thus handicap the modern brewing process, which is industrial and technological in character, and adversely affect the quality of the beer produced and also the economics of the overall process.

According to the present invention, there is provided a process for the production of beer wort, which comprises mashing unmalted grain, optionally in admixture with other extract-yielding material such that the proportion of unmalted grain is 20 to 100% by weight, based on the total weight of the extract-yielding material, in the presence of 0.004 to 5.0% by weight, based on the weight of unmalted grain, of the α -amylase obtained from *Bacillus subtilis* No. 24 ATCC 21813, the glucoamylase obtained from *Rhizopus nodosus* FER-P 1,635, the β -glucanase obtained from *Bacillus subtilis* No. 24 ATCC 21, 813, and at least one of the protease obtained from *Aspergillus melleus* IAM 2,066, the protease obtained from *Rhizopus niveus* IAM 6,035, and the protease obtained from *Aspergillus niger* IAM 2,020, the said enzymes being added either before or during the mashing.

The enzymes can be isolated from the respective micro-organism in manner known, *per se*, see, for example, J. Agr. Food Chem. 13 No. 1 30-34 (1965). "IAM" means Institute of Applied Microbiology, Tokyo.

The process of the present invention provides a particularly advantageous and reproducible equilibrium between saccharification and proteolysis which, because the individual enzymes are added separately, can be maintained for any of the various unmalted grain constituents, which can be used.

The proteases employed in the present invention degrade the protein present in the unmalted grain into fragments which are necessary for optimum performances of the brewing process and for the beer to have favourable qualities. Surprisingly, a relatively high proportion of the unmalted grain protein can be made available to the brewing process by the proteolytic degradation which occurs due to the action of the proteases. The amounts of certain aminoacids which are liberated, and the number, molecular size and polar properties of the polypeptides formed are especially important. The aminoacids which are liberated are particularly suitable for exerting a favourable influence on the metabolism of the pitching yeast in the sense of rapid and efficient fermentation of the pitching wort, with the individual aminoacids being assimilated or absorbed relatively quickly by the yeast.

According to J.S. McWilliam (J. Inst. Brew. 74 (1968) 38) the aminoacids which are liberated can be classified into three groups according to their rate of absorption and hence their biological importance in fermentation. As a result of the use of the particular proteases of the invention, not only are the aminoacids which are preferred in this context liberated from the unmalted grain proteins, but also the formation of vicinal diketones and of certain objectionable alcoholic compounds in the beer ultimately produced is substantially reduced. The polypeptides formed markedly assist foaming and foam stability and assist the colloidal stability of the beer, and the proteins responsible for low temperature cloudiness are degraded so extensively that the product has good storage stability.

As a result of the action of the α -amylase used in the invention, the saccharification of the starch contained in the unmalted grain, measured by the iodine normality method, can be carried out very rapidly and this results both in very favourable values of the extract content in the pitching wort, and in the preservation of a sufficiently high concentration of dextrins, required for foam stability. The glucoamylase, which is also employed in the invention, co-operates with the α -amylase so that even if the starch components of the unmalted grain are difficult to digest and the gelatinisation conditions are unfavourable, rapid saccharification of the unmalted grain and hence a general increase in the degree of fermentation can be achieved. Furthermore, the presence of the glucoamylase is particularly important in those cases where, in addition to starch, other carbohydrates based on glucose are also present in the unmalted grain.

The use, in the invention, of the β -glucanase enables the frequently lengthy clarification of the mash to be carried out over a much shorter period of time. Equally, the bright-filtering of the beer is greatly facilitated. Thus, the combined use of the proposed enzymes in the invention makes it possible for breweries using conventional brewing processes to produce, from unmalted grain, beer with good quality characteristics under economically favourable conditions.

The use of the enzymes of the invention makes it possible to carry out the customary brewing processes but with unmalted grain without undue difficulty. Examples of unmalted grain which can be used are, *inter alia*, fodder barley, rice, broken rice, rice grits, maize, maize grits, corn flakes and other cereals, and other raw materials containing starch and protein. In the decoction processes, the enzymatic digestion of the unmalted grain is carried out both in the partial mash (unmalted grain tub) and in the residual mash.

The percentage enzyme concentrations which should be added to the mash depend on the total proportion of unmalted grain in the extract-yielding material employed in the mash, as shown in the following Table:

Enzyme concentration dosage

	Proportion of unmalted grain	Protease	Enzyme concentration dosage		Glucoamylase	β -Glucanase
			I	II		
I	20% by weight	0.004% by weight	General use range	Preferred use range	0.004% by weight	0.004% by weight
	100% by weight	5.0% by weight			5.0% by weight	5.0% by weight
II	20% by weight	0.004% by weight	0.004% by weight	0.005% by weight	0.004% by weight	0.005% by weight
	100% by weight	0.7% by weight	1.0% by weight		0.7% by weight	1.0% by weight

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The enzymes are added after steeping and crushing the unmalted grain, and usually when making up the mash. The temperature of the mash at this stage is generally from 40° to 50°C.

The present invention is illustrated by the following Example.

Example

5 9.6 kg of fodder barley (protein content 11.1% by weight, moisture content 13.7% by weight, 5
extract content 63.0% by weight) and 6.0 kg of malt (protein content 11.2% by weight, moisture
content 4.9% by weight, extract content 75.5% by weight) are steeped in 45 l of steep liquor,
subsequently crushed and made up with water at a temperature of 45°C to 75 l of total mash. The
10 pH is 5.65. 26 l of this mixture are withdrawn as a partial mash and mixed with 5.8 kg of maize 10
grits (protein content 9.1% by weight, moisture content 13.7% by weight, extract content 78.6%
by weight). At 45°C, 3.0 g of the α -amylase obtained from *Bacillus subtilis* No. 24 ATCC 21,813
and 3.0 g of the β -glucanase obtained from *Bacillus subtilis* No. 24 ATCC 21,813 are added to the
15 partial mash and 7.5 g of the protease obtained from *Aspergillus melleus* IAM 2066 3.0 g of the 15
 α -amylase defined above, 7.5 g of the glucoamylase obtained from *Rhizopus noisus* FER-P 1,635
and 3.0 g of the β -glucanase defined above, are added to the residual mash. After completion of
the addition, the partial mash is warmed to 80°C over the course of 23 minutes while stirring,
20 stirred for 10 minutes at this temperature, then heated to 100° over the course of 14 minutes and 20
left at this temperature for 15 minutes. The residual mash in the mash tub, which has been stirred
in the meantime at a temperature of 45°C, is then mashed together with the partial mash to a
temperature of 63°C. The mash is left for 60 minutes. During this period, a vigorous
25 saccharification reaction commences. To complete this reaction, the temperature is raised to 75°C 25
in two steps and the mixture is stirred at this temperature until iodine normality is achieved. The
total period of mashing is 175 minutes.

The subsequent process steps such as production of the first wort, the wort boiling, the
25 addition of hops, the production of the pitching wort, the fermentation to give beer and the 25
subsequent maturing are carried out in accordance with customary brewing technology.

For comparison, a parallel experiment was carried out under the same conditions as described
above, but using high quality malt instead of the unmalted grain.

Assessment of the worts

30 I Unmalted grain decoction using the proposed enzymes, according to the invention 30
II Malt decoction

	I	II	
35 Viscosity of the first wort	low	normal	35
Mash yield	very high	high	
Pitching worts			
Final fermentation	very high	very high	
40 α -Amino-N	very high	high	40
Total N	very high	normal	
Lundin A	slight	normal	
45 B	normal	normal	45
C	high	normal	
Anthocyanogens	favourable	normal	
Yield of bitters	high	normal	
50 Course of fermentation	rapid	normal	50

The quantitative yield of aminoacids liberated proteolytically during mashing was substantially
higher in the unmalted grain decoction (see also Lundin C). Furthermore, the aminoacid
55 concentrations, which are important for the functioning of the pitching yeast, were also 55
advantageously distinctly higher than the values for the malt decoction.

In our copending Application No. 49563/73 (Serial No. 1442402), we describe and claim a
process for the production of beer wort from malt which is deficient in enzyme activity which
comprises mashing the malt in the presence of at least one of the protease obtained from
60 *Aspergillus melleus* IAM 2,066, the protease obtained from *Rhizopus niveus* IAM 6,035, the 60
protease, obtained from *Aspergillus niger* IAM 2,020, the α -amylase obtained from *Bacillus subtilis*
No. 24, ATCC 21,813, the glucoamylase obtained from *Rhizopus nodosus* FER-P 1,635 and the
 β -glucanase, obtained from *Bacillus subtilis* No. 24 ATCC 21,813, the said enzymes being added
before or during the mashing.

65 WHAT WE CLAIM IS:

The enzymes are added after steeping and crushing the unmalted grain, and usually when making up the mash. The temperature of the mash at this stage is generally from 40° to 50°C.

The present invention is illustrated by the following Example.

Example

- 5 9.6 kg of fodder barley (protein content 11.1% by weight, moisture content 13.7% by weight, extract content 63.0% by weight) and 6.0 kg of malt (protein content 11.2% by weight, moisture content 4.9% by weight, extract content 75.5% by weight) are steeped in 45 l of steep liquor, subsequently crushed and made up with water at a temperature of 45°C to 75 l of total mash. The pH is 5.65. 26 l of this mixture are withdrawn as a partial mash and mixed with 5.8 kg of maize
10 grits (protein content 9.1% by weight, moisture content 13.7% by weight, extract content 78.6% by weight). At 45°C, 3.0 g of the α -amylase obtained from *Bacillus subtilis* No. 24 ATCC 21,813 and 3.0 g of the β -glucanase obtained from *Bacillus subtilis* No. 24 ATCC 21,813 are added to the partial mash and 7.5 g of the protease obtained from *Aspergillus melleus* IAM 2066 3.0 g of the α -amylase defined above, 7.5 g of the glucoamylase obtained from *Rhizopus noibsus* FER-P 1,635
15 and 3.0 g of the β -glucanase defined above, are added to the residual mash. After completion of the addition, the partial mash is warmed to 80°C over the course of 23 minutes while stirring, stirred for 10 minutes at this temperature, then heated to 100° over the course of 14 minutes and left at this temperature for 15 minutes. The residual mash in the mash tub, which has been stirred in the meantime at a temperature of 45°C, is then mashed together with the partial mash to a
20 temperature of 63°C. The mash is left for 60 minutes. During this period, a vigorous saccharification reaction commences. To complete this reaction, the temperature is raised to 75°C in two steps and the mixture is stirred at this temperature until iodine normality is achieved. The total period of mashing is 175 minutes.

- 25 The subsequent process steps such as production of the first wort, the wort boiling, the addition of hops, the production of the pitching wort, the fermentation to give beer and the subsequent maturing are carried out in accordance with customary brewing technology.

For comparison, a parallel experiment was carried out under the same conditions as described above, but using high quality malt instead of the unmalted grain.

Assessment of the worts

- 30 I Unmalted grain decoction using the proposed enzymes, according to the invention 30
II Malt decoction

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Mash yield	very high	high	
Pitching worts			
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45 B	normal	normal	45
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Anthocyanogens	favourable	normal	
Yield of bitters	high	normal	
50 Course of fermentation	rapid	normal	50

- 55 The quantitative yield of aminoacids liberated proteolytically during mashing was substantially higher in the unmalted grain decoction (see also Lundin C). Furthermore, the aminoacid concentrations, which are important for the functioning of the pitching yeast, were also advantageously distinctly higher than the values for the malt decoction. 55

- 60 In our copending Application No. 49563/73 (Serial No. 1442402), we describe and claim a process for the production of beer wort from malt which is deficient in enzyme activity which comprises mashing the malt in the presence of at least one of the protease obtained from *Aspergillus melleus* IAM 2,066, the protease obtained from *Rhizopus niveus* IAM 6,035, the protease, obtained from *Aspergillus niger* IAM 2,020, the α -amylase obtained from *Bacillus subtilis* No. 24, ATCC 21,813, the glucoamylase obtained from *Rhizopus nodosus* FER-P 1,635 and the β -glucanase, obtained from *Bacillus subtilis* No. 24 ATCC 21,813, the said enzymes being added before or during the mashing. 60

- 65 WHAT WE CLAIM IS: 65





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1. Process for the production of beer wort, which comprises mashing unmalted grain, optionally in admixture with other extract-yielding material such that the proportion of unmalted grain is 20 to 100% by weight, based on the total weight of the extract-yielding material, in the presence of 0.004 to 5.0% by weight, based on the weight of the unmalted grain, of the α -amylase
5 obtained from *Bacillus subtilis* No. 24 ATCC 21813, the glucoamylase obtained from *Rhizopus nodosus* FER-P 1,635, the β -glucanase obtained from *Bacillus subtilis* No. 24 ATCC 21,813, and at least one of the protease obtained from *Aspergillus melleus* IAM 2,066, the protease obtained from *Rhizopus niveus* IAM 6,035, and the protease obtained from *Aspergillus niger* IAM 2,020, the said enzymes being added either before or during the mashing. 5
- 10 2. Process according to claim 1, wherein the protease is present in an amount of 0.004 – 0.7% by weight, the α -amylase is present in an amount of 0.005 – 1.0% by weight, the glucoamylase is present in an amount of 0.004 to 0.7% by weight and the β -glucanase is present in an amount of 0.005 – 1.0% by weight, based on the weight of unmalted grain. 10
- 15 3. Process according to claim 1 or 2, wherein the unmalted grain is fodder barley, rice, broken rice, rice grits, maize grits and/or cornflakes. 15
4. Process according to claim 1 substantially as described in the foregoing Example.
5. Beer wort produced by a process as claimed in any one of the preceding claims.

J.A. KEMP & CO.,
Chartered Patent Agents,
14 South Square,
Gray's Inn,
London WC1R 5EU.