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(54) Title: PROCESS FOR THE PREPARATION OF MALTED CEREALS

(57) Abstract

Process for the preparation of malted cereals, wherein the moistening step includes one or more stages until the material has a moisture content between 20 and 60% by weight, wherein after germination, the moistened cereals are preferably kilned by increasing the temperature to values between 40 and 150 °C until the material has a moisture content between 2 and 15% by weight, and wherein one or more microbial cultures selected from the group comprising one or more bacteria and/or one or more fungi, including moulds and yeasts, are added in one or more times either before or during the malting process of said cereals, and wherein at least one said microbial culture is inoculated by means of activated spores. Said activated spores are significantly more swollen than the dormant size, more particularly the size of the spores is increased by a factor preferably between 1.2 and 10 over the dormant sizeand/or having one or more germ tubes per spores.

PROCESS FOR THE PREPARATION OF MALTED CEREALS

Field of the invention.

The present invention is related to an improved process for the preparation of malted cereals, the improved malted cereals obtained and their use, especially in biotechnological processes for the preparation of beverages, or in food/feed applications, laundry and detergent systems and paper and pulp technology, as well as in bleaching applications.

Technological background of the invention.

Cereals such as barley, wheat, rye, corn, oats, rice, millet, triticale, and sorghum are used for the production of beverages. In most cases, they have been subjected to a malting process to take advantage of their increased enzymatic potential.

In traditional malting processes, the moisture content of cereals is raised either by immersion(s) and/or spraying(s), and the resulting high moisture content cereal is allowed to germinate. After reaching the proper physiological condition, it is preferably submitted to (a) drying step(s). In what follows the term steeping refers to the increase in moisture level while the term germination is used in the way it is in plant physiology. The drying operations are referred to as kilning and the term malting involves all operations needed to convert barley (or other cereals) to barley malts (or other cereal malts).

The quality of the malt obtained is, to a large extent, determined by the presence of plant endogenous enzymes generated during the malting process. For instance with cereals like barley used as a raw material for the malt production, the variety, the composition of the microbial flora and the environmental factors, such as agricultural practice, influence the quality of the malt. During cultivation and storage, cereals are contaminated with bacteria and fungi. In the malting plant, neither the air, the water nor the equipment are sterile, and the conditions of humidity, pH and temperature favour the growth of the microbial populations.

The variable cereal quality and the lack of means to make up for deficiencies during the malting process result in variability in malt quality. In many instances, this has to do with an imbalance of specific enzymatic potential and insufficient cell wall degradation. Apart from this, problems with microbial safety can occur. As a consequence of the defects in malt, quality problems occur in the production of beer, such as a poor filtration of the wort.

<u>State of the Art.</u>

During the malting of barley, the microflora develops and the quality of malt and beverages is influenced by the activity of the endogenous micro-organisms.

In analogy with other biotechnological processes, there have been attempts to optimise malt quality aspects by the addition of starter cultures during the malting process (Boivin, P. & Malanda, M., Influence of Starter Cultures in Malting on the Microflora Development and Malt Quality, EBC, Proceedings of the 24th Congress, pp. 95-102 (1993); Haikara, A. et al., Lactic Starter Cultures in Malting - A Novel Solution to Gushing Problems, EBC, Proceedings of the 24th Congress, pp. 163-172 (1993)).

Addition of spores of Geotrichum candidum to the steeping water results in the inhibition of the development of undesirable micro-organisms and in a decrease of the filtration time of wort made of the obtained malt. Treatment with Geotrichum candidum also inhibits the formation of mycotoxins by Fusarium spp.

The influence of Lactobacillus plantarum and Pediococcus pentosaceus has been tested on the microflora during malting and it has been found that these cultures act as natural preservatives as they restrict the growth of Fusarium and prevent gushing.

The international patent application WO94/29430 describes a process for improving the properties of malted cereals wherein starter cultures which comprise moulds, yeasts or bacteria are added prior and/or during malting of said cereals.

The preferred bacteria used are lactic acid producing bacteria such as various Lactobacilli, e.g.

Lactobacillus casei, Lactobacillus casei var rhamnosus, Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus brevis, and bacteria of the genus Pediococcus, e.g. Pediococcus acidilactici.

Preferred moulds are moulds of the genus Aspergillus and Geotrichum, like Geotrichum candidum.

The international patent application WO94/16053 describes a process for treating cereals for inhibiting growth of unwanted microbial species by inoculating the cereals during the germination process with a lactic acid bacteria preparation or a preparation produced by lactic acid bacteria. The preferred bacteria are lactic acid bacteria belonging to genus Lactococcus, Leuconostoc, Pediococcus or Lactobacillus.

The British patent application GB-1211779 provides a method for the automatic control and regulation of a malting process. It enables one to determine the parameters necessary for a successful automatically controlled and regulated malting process.

In the Proceedings of the European Brewery Convention, volume 16, 1977, pages 245 to 254, the influence of some fungi on malt quality is described, more specifically, contamination of barley malt with fungi which has led to gushing and other qualitative changes in the beer. Reference is also made to spores of these fungi.

The German patent application DE-3028360 discloses a method to make malt out of corn.

However, malt prepared according to the present invention is of better quality than that prepared according to any of the previous documents. This is exemplified by higher β-glucanase and xylanase activities, lower β-glucan contents in malt and wort and improved

European Brewery Convention analytical data.

Aims of the invention.

The present invention aims to provide an improved preparation process for malted cereals and improved malted cereals.

A main aim of the invention is to provide an improved preparation process for malted cereals and improved malted cereals in terms of brewing performances, especially malted cereals having an improved quality in terms of enzymatic potential and microbial safety.

Another aim is to provide a process and improved malted cereals which vary less in quality with the raw material used.

A further aim of the invention is to obtain malted cereals which improve the biotechnological production process of beverages and may improve the properties of the said obtained beverages.

Another aim of the invention is to use malted cereals with improved properties in food technology such as the bakery industry as a bread additive, in the feed technology for the production of high efficiency animal feed, in the paper and pulp technology, as a bleaching agent, or in laundry and detergent systems such as laundry liquids, laundry powders, dish-washing liquids and powders, softeners, cleaners and soap bars as a source for enzymatic cleaning agents.

Summary of the invention.

The present invention is more specifically related to a process for the preparation of malted cereals, wherein the steeping step includes one or more wetting stages at a temperature between 5 and 30 °C, preferably between 10 and 20 °C, until the material has a moisture content between 20 and 60% by weight, preferably between 38 and 47%, wherein after a germination period between 2 and 7 days, preferably between 3 to 6 days at a temperature between 10 and 30 °C, preferably between 14 and 18 °C, the steeped and germinated cereals are preferably kilned by increasing the temperature to values between 40 and 150 °C, preferably between 45 and 85 °C, until the material has a moisture content between 2 and 15% by weight, preferably between 4 and 7%, and wherein one or more microbial cultures selected from the group consisting of one or more bacteria and/or one or more fungi are added in one or more times either before or during or after the malting process of said cereals, and wherein at least one of said microbial cultures is inoculated by means of activated spores, said activated spores being significantly more swollen than the dormant size, the size of the spores being increased by a factor preferably between 1.2 and 10 over the dormant size and/or having one or more germ tubes per spore.

The term "fungi" as used in the present application includes both moulds and yeasts.

This process, thus, allows for a broad flexibility in malting conditions.

Preferably, for the preparation of malted barley, said bacteria are selected from the group comprising Micrococcus spp., Streptococcus spp., Leuconostoc spp., Pediococcus spp. preferentially Pediococcus halophilus, Pediococcus cerevisiae, Pediococcus damnosus, Pediococcus hemophilus, Pediococcus parvulus, Pediococcus soyae, Lactococcus spp., Lactobacillus spp. preferentially Lactobacillus acidophilus, Lactobacillus amylovorus, Lactobacillus bavaricus, Lactobacillus bifermentans, Lactobacillus brevis var lindneri, Lactobacillus casei var casei, Lactobacillus delbrueckii, Lactobacillus delbrueckii var lactis, Lactobacillus delbrueckii var bulgaricus, Lactobacillus fermenti, Lactobacillus gasserii, Lactobacillus helveticus, Lactobacillus hilgardii, Lactobacillus renterii, Lactobacillus saké, Lactobacillus sativorius, Lactobacillus cremoris, Lactobacillus kefir, Lactobacillus pentoceticus, Lactobacillus cellobiosus, Lactobacillus bruxellensis, Lactobacillus buchnerii, Lactobacillus coryneformis, Lactobacillus confusus, Lactobacillus florentinus, Lactobacillus viridescens, Corynebacterium spp., Propionibacterium spp., Bifidobacterium spp., Streptomyces spp., Bacillus spp., Sporolactobacillus spp., Acetobacter spp., Agrobacterium spp., Alcaligenes spp., Pseudomonas spp. preferentially Pseudomonas amylophilia, Pseudomonas aeruginosa, Pseudomonas cocovenenans, Pseudomonas mexicana, Pseudomonas pseudomallei, Gluconobacter spp., Enterobacter spp., Erwinia spp., Klebsiella spp., Proteus spp.

Preferably, for the preparation of malted barley the fungi are selected from the group (genera as described by Ainsworth and Bisby's dictionary of the fungi, 8th edition, 1995, edited by DL Hawksworth, PM Kirk, BC Sutton, and Debit-note Pegler (632 pp) Cab International) comprising Ascomycota preferentially Dothideales preferentially Mycosphaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericilla spp., Euroteum spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp.; Hypocreales preferentially Hypocreceae preferentially Hypocrea spp.; Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccharomycodaceae preferentially Hanseniaspora spp.; Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp.; Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Chlamydomucor spp., Mucor spp. preferentially Mucor circinelloides, Mucor grisecyanus, Mucor hiemalis, Mucor indicus, Mucor mucedo, Mucor piriformis, Mucor plumbeus, Mucor praini, Mucor pusillus, Mucor silvaticus, Mucor javanicus, Mucor racemosus, Mucor rouxianus, Mucor rouxii, Mucor aromaticus, Mucor flavus, Mucor miehei, Rhizopus spp. preferentially Rhizopus arrhizus, Rhizopus oligosporus, Rhizopus oryzae preferentially strains ATCC 4858, ATCC 9363, NRRL 1891, NRRL 1472, Rhizopus stolonifer, Rhizopus thailandensis, Rhizopus formosaensis, Rhizopus chinensis, Rhizopus cohnii, Rhizopus japonicus, Rhizopus nodosus, Rhizopus delemar, Rhizopus acetorinus, Rhizopus chlamydosporus, Rhizopus circinans, Rhizopus javanicus, Rhizopus peka, Rhizopus saito, Rhizopus tritici, Rhizopus niveus, Rhizopus microsporus; Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp. preferentially Monilia candida, Monilia sitophila, Mycoderma spp., Candida spp. preferentially Candida diddensiae, Candida edax, Candida etchellsii, Candida kefir, Candida krisei, Candida lactosa, Candida lambica, Candida melinii, Candida utilis, Candida milleri, Candida mycoderma, Candida parapsilosis, Candida obtux, Candida tropicalis, Candida valida, Candida versatilis, Candida guilliermondii, Rhodotorula spp., Torulopsis spp., Geotrichum spp. preferentially Geotrichum amycelium, Geotrichum armillariae, Geotrichum asteroides, Geotrichum bipunctatum, Geotrichum dulcitum, Geotrichum eriense, Geotrichum fici, Geotrichum flavo-brunneum, Geotrichum fragrans, Geotrichum gracile, Geotrichum heritum, Geotrichum klebaknii, Geotrichum penicillatum, Geotrichum hirtum, Geotrichum pseudocandidum, Geotrichum rectangulatum, Geotrichum suaveolens, Geotrichum vanryiae, Geotrichum loubieri, Geotrichum microsporum, Cladosporium spp., Trichoderma spp. preferentially Trichoderma hamatum, Trichoderma harzianum, Trichoderma koningii, Trichoderma pseudokoningii, Trichoderma reesei, Trichoderma virgatum, Trichoderma viride, Oidium spp., Alternaria spp. preferentially Alternaria alternata, Alternaria tenuis, Helminthosporium spp. preferentially Helminthosporium gramineum, Helminthosporium sativum, Helminthosporium teres, Aspergillus spp. as described by R.A. Samson ((1994) in Biotechnological handbooks, Volume 7 : Aspergillus, edited by Smith, J.E. (273 pp), Plenum Press) preferentially Aspergillus ochraseus Group (Thom & Church), Aspergillus nidulans Group (Thom & Church), Aspergillus versicolor Group (Thom & Church), Aspergillus wentii Group (Thom & Raper), Aspergillus candidus Group (Thom & Raper), Aspergillus flavus Group (Raper & Fennell), Aspergillus niger Group (Thom & Church), Penicillum spp. preferentially Penicillum aculeatum, Penicillum citrinum, Penicillum claviforme, Penicillum funiculosum, Penicillum italicum, Penicillum lanoso-viride, Penicillum emersonii, Penicillum lilacinum, Penicillum expansum.

Preferably, for the preparation of malted cereals other than malted barley, especially for the preparation of malted wheat, rye, corn, oats, rice, millet, triticale, and sorghum, said bacteria are selected from the group comprising Micrococcus spp., Streptococcus spp., Leuconostoc spp., Pediococcus spp., Lactococcus spp., Lactobacillus spp., Corynebacterium spp., Propionibacterium spp., Bifidobacterium spp., Streptomyces spp., Bacillus spp., Sporolactobacillus spp., Acetobacter spp., Agrobacterium spp., Alcaligenes spp., Pseudomonas spp., Gluconobacter spp., Enterobacter spp., Erwinia spp., Klebsiella spp., Proteus spp. or a mixture thereof; and said fungi are fungi selected from the group consisting of : Ascomycota preferentially Dothideales preferentially Mycophaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericilla spp., Euroteum spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp., Hypocreales preferentially Hypocreaceae preferentially Hypocrea spp., Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccaromycodaceae preferentially Hanseniaspora spp., Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp., Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Clamydomucor spp., Mucor spp., Rhizopus spp.; Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp., Mycoderma spp., Candida spp., Rhodotorula spp., Torulopsis spp., Geotrichum spp., Cladosporium spp., Trichoderma spp., Oidium spp., Alternaria spp., Helminthosporium spp., Aspergillus spp., Penicillium spp.

According to a preferred embodiment, the preparation process of malted cereals according to the invention comprises the following steps: the steeping step includes one or more wetting stages or the total time of submersion in water during steeping for physiological reasons does not exceed 30 hours (preferably 10 to 25 hours) or the kilning step includes more than two temperature steps and the microbial cultures which are added, are preferably selected from the group consisting of Rhizopus spp., preferably Rhizopus oryzae such as Rhizopus oryzae strain ATCC9363 and/or Pseudomonas spp., preferably Pseudomonas herbicola, or Aspergillus spp., preferably Aspergillus oryzae such as Aspergillus oryzae strain ATCC14156.

According to the invention, the malted cereals are selected from the group comprising barley, wheat, rye, corn, oats, rice, millet, triticale, and sorghum.

In the process according to the invention, the same or different microbial cultures in the presence of activated spores are added in one or more time(s). The microbial cultures used are preferably fungal cultures. The use of activated spores greatly enhances their contribution to improved malt quality, most likely because of more vigorous growth. The activated spores have one of the following properties: the treated spores are more swollen than their dormant size, more particularly, the size of the spores is increased by a factor preferably between 1.2 and 10 over their dormant size and/or one or more germ tubes per spore are formed. The activated spores are prepared by subjecting them to environmental changes, preferably by at least one or a combination of the following treatments:

(a) cycles of wetting and/or drying;

(b) addition of appropriate nutritional supplies (such as a nitrogen source, preferably amino acids and/or a carbon source, preferably mono- or disaccharides) or spore elements;

(c) exposure to temperature changes, preferably within a temperature range of 0 to 80°C;

(d) exposure to changes in pH, preferably within a pH range of 2.0 to 8.0, more preferably between 3.0 and 6.0.

The specialist may easily select precise treatment steps to obtain either swelling of the spores or germ tubes as above-mentioned.

The present invention also concerns the malted cereals obtained, which present improved analysis results according to European Brewery Convention. Said improvements may have to do with modification and/or increased hydrolytic enzyme activities. At the same time, a decreased level of toxins, an increased microbial safety by e.g. outcompeting undesirable microbial flora such as Fusarium and/or an increased acceptability compared to the malted cereals according to the state of the art, may be observed.

For instance, the malted cereals according to the invention may have a lower ß-glucan content or a higher β-glucanase or xylanase activity (as represented in the following examples and figures) than the malted cereals according to the state of the art. This allows for a better processability of the malt in wort and beer production as exemplified by increased rates of filtration.

Another object of the present invention concerns the use of the malted cereals according to the invention for the preparation of beverages.

The invention is also related to these improved beverages. The improved malted cereals according to the invention can also be advantageously used during brewing of alcohol free or low alcohol beer or light beer since the higher enzymatic activity will enhance removal of the alcohol from the beer.

The improved malted cereals according to the invention could also be used in other biotechnological processes well known by the Man Skilled in the Art, in which in most cases advantage is taken of their improved quality.

Another object of the present invention concerns the use of the malted cereals with improved properties in food technology such as the bakery industry as a bread additive, in the feed technology for the production of animal feed with higher conversion characteristics, in paper and pulp technology, as a bleaching agent, or in detergent compositions.

The present invention will be further described in various examples in view of the following drawings.

Brief description of the drawings.

Figure 1 represents the β-glucanase activity of malted barley obtained according to the preparation process of example 1. (legend: see example 1)

Figure 2 represents the xylanase activity of malted barley obtained according to the preparation process of example 1. (legend: see example 1)

Figure 3 represents the β-glucanase activity of malted barley obtained according to the preparation process of example 3.(legend: see example 3)

Figure 4 represents the xylanase activity of malted barley obtained according to the preparation process of example 3. (legend: see example 3)

Figure 5 represents the relative increase factor (R.I.F.) for bacterial populations (see text, malt evaluation, example 2) (legend: see example 2)

Example 1.

1. Preparation of microbial cultures

Strain

- S46 : Rhizopus oryzae ATCC 9363

Preparation of the spore suspension

- the strain was grown on PDA (Potato Dextrose Agar, Oxoid) for approximately 10 days at 28 °C;
- the spores were harvested by flooding the cultures with sterile physiological saline (0.9% NaCl) and by rubbing the sporulated mycelium gently with a sterile spatula;
- the spore suspension was washed twice with sterile physiological saline (0.9% NaCl) by centrifugation (5500 rpm, Sorvall type SS-34 ®, for 15 min) and resuspended in sterile physiological saline (0.9% NaCl);
- the spore density was determined microscopically using a Thoma counting chamber.

Activation of the spore suspension

- 10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid), pH = 4.0 and incubated in a shaking water bath during 5 to 6 hours at ± 42 °C;
- the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-34 ®, for 15 min), washed once with sterile physiological saline (0.9% NaCl) by centrifugation (3500 rpm, Sorvall type SS-34 ®, for 15 min) and resuspended in sterile physiological saline (0.9% NaCl).

2. Barley

- Plaisant - 1994 French harvest

3. Process

Setup

Malts were made by four different malting processes :

- *A1. traditional malting* (without inoculation of any spore suspension)
- *B1. malting with inoculation with non-activated spores* (inoculation of the steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363)
- *C1. malting process according to the invention* (inoculation of the steeped barley with a suspension of activated spores of Rhizopus oryzae ATCC 9363)
- *D1. malting process according to the invention* (inoculation of the steeped barley during the first wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

Steeping

- the steeping was carried out on a 2 kg base with a total water (tap water) to air dry barley ratio of 1.5:1;
- use was made of 2 fermentors (Bioflo III, New Brunswick Scientific), in which perforated plates were placed;
- temperature was only controlled during the wet stages; during the air rest stages the system was allowed to reach room temperature (± 20 °C);
- during the whole steeping period the barley was aerated (4 liter sterile air per minute);
- steeping was carried out by immersion using the following scheme :

	Temperature (°C)	Duration (h)
First wet stage	13	6:00
First air rest stage	20	17:00
Second wet stage	14	5:00
Second air rest stage	20	15:30
Third wet stage	16	2:30

Addition of the microbial cultures

- ± 460 g of steeped barley was immersed in 0.5 l of tap water which contained no spores (A1), non-activated spores of Rhizopus oryzae ATCC 9363 (B1) or activated spores of Rhizopus oryzae ATCC 9363 (C1, according to the invention); for B1 and C1, the steeped barley was inoculated with 10^4 spores per gram of air dry barley;
- during the steeping, 10^4 activated spores per gram air dry barley were inoculated to the water of the first wet stage (D1);
- the fluid was removed by draining.

Germination

- germination was carried out in a cylindrical container with perforated lids at a temperature of 16-18 °C during 4 days;
- air was supplied by natural diffusion;
- the containers were slowly rotated on an electronically controlled roller system (Cellroll ®, Tecnorama); i.e. every two hours the containers were rolled for 15 min at 1 rpm.

Kilning

- the kilning was carried out in a Joe White malting unit (Australia)

	Air flow (%)	Recirc. Air (%)	Temp. (°C)	Durat. (h)
First kilning stage	25	0	62	3:00
Second kilning stage	25	0	65	2:00
Third kilning stage	25	0	68	2:00
Fourth kilning stage	25	25	73	2:00
Fifth kilning stage	25	50	78	1:00
Sixth kilning stage	25	75	80	2:00
Seventh kilning stage	25	100	83	6:00
Shut down air off				Time-out

4. Methods of analysis and results

Methods for determination and units of moisture, extract, extract difference, color, total protein content, soluble protein content, Kolbach index, pH, diastatic power, according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

Methods for determination and units of turbidity, friability, homogeneity, whole grains, β-glucan content, according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau, supplement published in 1989).

Postcoloration of the wort is determined after boiling the congress wort under reflux at 108 °C during 2 hours.

The viscosity of the congress wort is determined with the Delta-viscosimeter.

For the determination of the filtration volume, the congress wort is filtered over a Schleicher and Schuell 597 1/2 folded filter. The volume (in ml) that is obtained after 1 hour of filtration is the filtration volume of the wort.

Modification is determined with the Calcofluor apparatus (Haffmans) according to the Carlsberg method (Analytica-European Brewery Convention, Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

The β-glucanase and xylanase activities are determined with the β-glucazym method ((Megazyme (Austr.) Pty Ltd (April, 1993)) and the xylazym method ((Megazyme (Austr.) Pty Ltd (September, 1995)), respectively.

	Traditional malting process (A1)	Malting process with inoculation with non-activated spores (B1)	Malting process according to the invention (C1)	Malting process according to the invention (D1)
Moisture	3.9	4.1	3.8	4.3
Extract	80.3	80.4	80.3	79.8
Extract difference	0.8	0.8	0.4	1.1
Color	3.3	3.3	4.1	4.1
Wort turbidity	1.3	1.2	0.7	0.8
Postcoloration	6.0	6.0	7.3	7.5
Total protein content	10.1	10.3	10.0	10.1
Soluble protein content	4.1	4.4	4.8	5.2
Kolbach index	40.6	42.7	48.0	51.0
Viscosity	1.57	1.52	1.52	1.54
pH	6.05	6.3	5.87	5.79
Diastatic power	345	349	352	419
Whole grains	0.3	0.3	0.1	ND
Friability	83	82	83.9	ND
Homogeneity	98.5	97.9	98.6	ND
β-glucan content	122	108	46	<40
Filtration volume	210	265	290	275
Modification	88.2	90.5	93.4	ND
β-glucanase	214	371	683	3856
Xylanase activity	28	34	56	984

ND: not determined

Figures 1 and 2 represent the β-glucanase and xylanase activity, respectively of the obtained malted barley (A1, B1, C1, D1). The β-glucanase activity was determined with the β-glucazyme method [Megazyme (Austr.) Pty Ltd. (April, 1993)]. Therefore, malt β-glucanase activity (U/kg) was calculated as 380 x E (590 nm) + 20. The xylanase activity was determined with the endo 1-4-xylazyme method [Megazyme (Austr.) Pty Ltd. (September 1995)] Therefore, malt xylanase activity (U/kg) was calculated as (46.8 x E (590nm)+0.9)x 5.

Example 2

1. Preparation of microbial cultures

Strain

- S46 : Rhizopus oryzae ATCC 9363

Preparation of the spore suspension

- as described in example 1

Activation of the spore suspension

- as described in example 1

2. Barley

- Stander - 1995 North American harvest

3. Process

Setup

Malts were made by six different malting processes :

- *A2. traditional malting process*
  (without inoculation of any spore suspension)

- *B2. malting process with inoculation with non-activated spores* (inoculation of the steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363)
- *C2. malting process according to the invention* (inoculation of the steeped barley during the first wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)
- *D2. malting process according to the invention* (inoculation of the steeped barley during the second wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)
- *E2. malting process according to the invention* (inoculation of the steeped barley during the third wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)
- *F2. malting process according to the invention* (inoculation of the steeped barley with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

Steeping and addition of the microbial cultures

- the steeping was carried out on a 300 g base with a total water (tap water) to air dry barley ratio of 5:3;
- use was made of 2000 ml flasks;
- a temperature of 18 ^{O}C was maintained during the wet stages and during the air rest stages;
- during the whole steeping period the barley was aerated by means of compressed air;

- steeping was carried out by immersion using the following schedule :

	Duration (h)
First wet stage	6:00
First air rest stage	18:00
Second wet stage	5:00
Second air rest stage	19:00
Third wet stage	2:00

- during the steeping, 10^4 activated spores per gram of air dry barley were inoculated to the water of the first wet stage (C2), of the second wet stage (D2) or of the third wet stage (E2) before immersion of the barley;
- the steeped barley was immersed in 0.5 litre of tap water which contained no spores (A2, C2, D2, E2), non-activated (B2) or activated (F2) spores;
- for B2, and F2, the steeped barley was inoculated with 10^4 spores per gram of air dry barley;
- the fluid was removed by draining.

<u>Germination</u>

- as described in example 1

<u>Kilning</u>

- as described in example 1

<u>Malt evaluation</u>

<u>Determination of the increase of the bacterial population</u>

To judge the evolution of the bacterial population during the malting process, a relative increase factor (R.I.F.) was determined by dividing the total bacterial count occurring on the green malt by the total bacterial count occurring on the barley. The total bacterial count was determined after plating appropriate dilutions of an extract of the kernels on Tryptic Soy Agar (Oxoid) supplemented with 100 ppm pimaricine and after incubation at 28 °C for 3 days.

Figure 5 shows the increase of the bacterial population during the malting according to the preparation process of example 2.

Example 3

1. Preparation of microbial cultures

Strain

- S46 : Rhizopus oryzae ATCC 9363

Preparation of the spore suspension

- as described in example 1

Activation of the spore suspension

- as described in example 1

2. Barley

- Plaisant - 1994 French harvest;

3. Process

Setup

Malts were made by three different malting processes :

- *A3 traditional malting*
  (without inoculation of any spore suspension)

- *B3 malting process with inoculation with non-activated spores*
  (inoculation of the steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363)
- *C3 malting process according to the invention*
  (inoculation of the steeped barley with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

<u>Steeping</u>

- the steeping was carried out on a 2 kg base air dry barley with a total water (tap water) to air dry barley ratio of 1.5:1;
- the pH of the steeping water was controlled at pH = 5.5 by addition of lactic acid and NaOH;
- a fermentor (Bioflo III, New Brunswick Scientific), in which a perforated plate was placed, was used for steeping;
- temperature was only controlled during the wet stages; during the air rest stages the system was allowed to reach room temperature (ca.20 °C);
- during the whole steeping period the barley was aerated (4 liter sterile air per minute);
- steeping was carried out by immersion using the following schedule :

	Temperature (^oC)	Duration (h)
First wet stage	13	6:00
First air rest stage	20	17:00
Second wet stage	14	5:00
Second air rest stage	20	15:30
Third wet stage	16	2:30

Addition of the microbial cultures

- 460 g of steeped barley was immersed in 0.5 l of tap water which contained no spores (A3), non-activated spores of Rhizopus oryzae ATCC 9363 (B3) or activated spores of Rhizopus oryzae ATCC 9363 (C3 according to the invention); for B3 and C3, the steeped barley was inoculated with 1.10^4 spores per gram of air dry barley;
- the fluid was removed by draining.

Germination

- as described in example 1

Kilning

- as described in example 1

4. Methods of analysis and results

These were as described in example 1 (4. Methods of analysis and results).

See table on next page. In this table :

* A1/3 : Traditional malting process

  B1/3 : Malting process with inoculation with non-activated spores

  C1/3 : Malting process according to the invention

	Example 3 pH control of the steeping water (pH = 5.5)			Example 1 No pH control of the steeping water		
	A3	B3	C3	A1	B1	C1
Moisture	3.8	3.6	3.7	3.9	4.1	3.8
Extract	78.9	80.2	80.7	80.3	80.4	80.3
Extract difference	0.6	0.7	0.4	0.8	0.8	0.4
Color	3.2	4.2	4.4	3.3	3.3	4.1
Wort turbidity	1	1	0.8	1.3	1.2	0.7
Postcoloration	5.1	7	7.2	6	6	7.3
Total protein content	10.2	10.1	10	10.1	10.3	10
Soluble protein content	4	4.4	4.8	4.1	4.4	4.8
Kolbach index	39.2	43.6	48	40.6	42.7	48
Viscosity	1.52	1.53	1.52	1.57	1.52	1.52
pH	6.02	5.97	5.91	6.05	6.03	5.87
Diastatic power	348	333	355	345	349	352
Whole grains	0.2	0.2	0.1	0.3	0.3	0.1
Friability	81	81	85	83	82	83.9
Homogeneity	97.6	97.8	98.9	98.5	97.9	98.6
β-glucan content	190	57	40	122	108	46
Filtration volume	210	215	200	210	265	290
Modification	84.1	85.5	87.4	88.2	90.5	93.4
β-glucanase activity	202	931	1322	214	371	683
Xylanase activity	43	65	71	28	34	56

Figure 3 represents the ß-Glucanase activity, measured according to ß-Glucazyme method [Megazyme (AUSTR) Pty. Ltd.] of the malted cereals A3, B3 and C3. Malt β-glucanase activity (U/kg) was calculated as described in example 1. A3 was obtained by the traditional malting process with pH control of the steeping water (pH = 5.5). B3 resulted from the malting process according to the invention with the inoculation of steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363 and with pH control of the steeping water (pH = 5.5). C3 was obtained by the malting process according to the invention with the inoculation of the steeped barley with a suspension of activated spores of Rhizopus oryzae ATCC 9363 and with pH control of the steeping water (pH = 5.5).

These results show the increased β-glucanase activity when the pH of the steeping water is maintained at around 5.5.

Figure 4 gives the corresponding results for xylanase activity. These were measured according to Xylazyme method, Megazyme ((AUSTR), Pty. Ltd. (September 1995)). Malt xylanase activity was calculated as described in example 1.

Comparison of the β-glucanase activity obtained according to examples 1 and 3 with the β-glucanase activity according to the state of the art as described in WO94/29430

In order to compare the improved results regarding β-glucanase activity by the present invention, we defined the factor μ as follows:

$$\mu = \frac{\beta\text{-glucanase activity of the treated malt}}{\beta\text{-glucanase activity of the control malt}}$$

This factor was calculated for control malt and malt treated with Rhizopus oryzae ATCC 9363 as described in examples 1 and 3 of the present invention.

It was also calculated for the data described in WO94/29430 (example 1) where Geotrichum candidum was used.

Both as described in WO94/29430, and in the present application, β-glucanase activity was determined with the beta-glucazyme method [Megazyme (Austr) Pty. Ltd. (April 1993)]. Therefore, malt β-glucanase activity (U/kg) was calculated as 380 x E(590 nm) + 20 and one unit of activity was defined as the amount of enzyme required to release one micromole of reducing sugar equivalents per minute under the defined above conditions.

Comparison of the results:

State of the art				Invention			
	μ	μ	μ	Ex. 1	μ	Ex. 3	μ
Gc *	1.48	Gc *	1.98	C1/A1	3.19	C3/A3	6.54
B1/A1	1.73	B3/A3	4.61	D1/A1	18.02		

*Gc : Geotrichum candidum

The results clearly show that the present invention provides for a more drastic increase in malt β-glucanase activity than that described earlier (WO 94/29430).

It thus appears that it is possible to obtain malted cereals having a β-glucanase activity increased by at least a factor 4 compared to the conventional malting process wherein the addition of microbial culture is omitted.

From figure 2 and 4, it also appears that it is possible to obtain malted cereals having a xylanase activity increased by at least a factor 4 compared to conventional malting process wherein the addition of microbial culture is omitted.

Example 4

1. Preparation of the microbial cultures

Strain

- S40: *Aspergillus oryzae* ATCC 14156

Preparation of the spore suspension

- the strain was grown on PDA (Potato Dextrose Agar, Oxoid) for approximately 7 days at 28 °C;
- the spores were harvested by flooding the culture with sterile physiological saline (0.9% NaCl) and by rubbing the sporulated mycelium gently with a sterile spatula;
- the spore supension was washed once with sterile physiological saline (0.9% NaCl) by centrifugation (5500 rpm, Sorvall type SS-34®, for 15 min) and resuspended in sterile physiological saline (0.9% NaCl);
- the spore density was determined microscopically using a Thoma counting chamber.

Activation of the spore suspension

- 5.10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid), pH = 5.0 and incubated in a shaking water bath during 3 hours (1) or 1 hour (2) at 35 °C;

2. Cereal

- Clarine barley - 1995 French harvest

3. Process

Setup

Malts were made by two different malting processes :

- A4. traditional malting
- (without inoculation of any spore suspension)
- E4. malting process according to the invention
- (inoculation of the steeped barley during the first and third wet stage with a suspension of activated spores of Aspergillus oryzae ATCC 14156)

Steeping

- as described in example 1

Addition of the microbial cultures

- during the steeping, 5.10^3 activated spores (1) per gram air dry barley were inoculated to the water of the first wet stage and 1.10^4 activated spores (2) per gram air dry barley were inoculated to the water of the third wet stage (E4);

Germination

- germination of ± 460 g steeped barley was carried out in cylindrical containers with perforated lids at a temperature of 16-18 °C during 4 days;
- air was supplied by natural diffusion;

- the containers were slowly rotated on an electronically controlled roller system (Cellroll ®, Tecnorama); i.e. every two hours the containers were rolled for 15 min at 1 rpm.

Kilning

- as described in example 1

4. Methods of analysis and results

These were as described in example 1 (4. methods of analysis and results)

Acrospire lengths were determined by classifying kernels into 6 categories, i.e. those with kernel having no acrospire (O) and those having acrospire length of 0 to 25% (0 - 1/4), 25 to 50% (1/4 - 1/2), 50 to 75% (3/4 - 1) and > 100% (>1) of the kernel length.

		0	0 - 1/4	1/4 - 1/2	1/2 - 3/4	3/4 - 1	> 1
1 day germination	A4	0	1	60	39	0	0
1 day germination	E4	0	0	11	77	12	0
4 days germination	A4	1	1	31	64	3	0
4 days germination	E4	1	0	1	42	49	7

It was noticed that the use of activated spores of Aspergillus oryzae ATCC 14156 improved the malt analytical specifications (cf. infra). Furthermore, it was unexpectedly found that during the malting process the barley acrospire lengths were significantly longer when the process according to the invention rather than the traditional process was used.

	Traditional malting process (A4)	Malting process according to the invention (E4)
Moisture	4.3	4.0
Extract	80.9	81.1
Extract difference	1.0	0.3
Color	2.8	3.2
Wort turbidity	1.6	1.0
Postcoloration	4.8	5.4
Total protein content	10.1	10.0
Soluble protein content	3.9	4.5
Kolbach index	38.6	44.7
Viscosity	1.57	1.48
pH	5.98	5.89
Diastatic power	197	201
Whole grains	1.3	0.6
Friability	81	89
Homogeneity	95.0	98.4
β-glucan content	378	132
Filtration volume	300	310
Modification	83.9	89.8
β-glucanase activity	309	392
Xylanase activity	27.82	17.52

Example 5

1. Preparation of the microbial cultures

Strains

- S40: Aspergillus oryzae ATCC 14156

- S46 : Rhizopus oryzae ATCC 9363

Preparation of the spore suspensions

- as described in example 4

Activation of the spore suspensions

S40 :

- 5.10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid), pH = 5.0 and incubated in a shaking water bath during 1 hour at 35 °C;
- the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-34®, for 15 min) and resuspended in sterile physiological saline (0.9% NaCl)

S46 :

- 5.10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid), pH = 4.0 and incubated in a shaking water bath during 5 hours at 42 °C;
- the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-34®, for 15 min) and resuspended in sterile physiological saline (0.9% NaCl)

2. Cereal

- Clarine - 1995 French harvest

3. Process

Setup

Malts were made by two different malting processes :

- A5. traditional malting
- (without inoculation of any spore suspension)
- F5. malting process according to the invention
- (inoculation of the steeped barley during the first wet stage with a suspension of activated spores of Aspergillus oryzae ATCC 14156 and after steeping with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

Steeping

- as described in example 1

Addition of the microbial cultures

- during steeping, 1.10^4 activated spores of *Aspergillus oryzae* ATCC 14156 per gram air dry barley were inoculated to the water of the first wet stage (F5, according to the invention);
- ± 460 g of steeped barley was immersed in 0.5 l of tap water which contained no spores (A5) or activated spores of *Rhizopus oryzae* ATCC 9363 (F5, according to the invention); for F5 the steeped barley was inoculated with 1.10^4 activated spores per gram air dry barley;
- the fluid was removed by draining;

Germination

- as described in example 4

Kilning

- as described in example 1

4. Methods of analysis and results

These were as described in example 1 (4. methods of analysis and results)

Method for the determination of the acrospire length as in example 4.

		0	0 - 1/4	1/4 - 1/2	1/2 - 3/4	3/4 - 1	> 1
1 day germination	A5	1	1	53	44	1	0
1 day germination	F5	0	1	21	73	5	0
4 days germination	A5	0	0	0	29	63	8
4 days germination	F5	0	0	0	13	63	24

	Traditional malting process (A5)	Malting process according to the invention (F5)
Moisture	3.9	4.2
Extract	81.4	81.8
Extract difference	0.9	1.1
Color	3.8	3.8
Wort turbidity	1.4	1.0
Postcoloration	6.9	6.4
Total protein content	10.1	10.2
Soluble protein content	4.8	5.2
Kolbach index	48.0	51.3
Viscosity	1.51	1.50
pH	5.88	5.82
Diastatic power	199	214
Whole grains	0.8	1.1
Friability	89	95
Homogeneity	98.3	98.3
b-glucan content	120	51
Filtration volume	270	220
Modification	96.8	98.6
β-glucanase activity	263	907
Xylanase activity	28.86	57.76

<u>Example 6</u>

<u>1. Preparation of the microbial cultures</u>

<u>Strain</u>

- S46: Rhizopus oryzae ATCC 9363

Preparation of the spore suspension

- as described in example 4

Activation of the spore suspension

- 5.10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid), pH = 4.0 and incubated in a shaking water bath during 5 hours at 42 °C;
- the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-34®, for 15 min) and resuspended in sterile physiological saline (0.9% NaCl)

2. Cereal

- wheat: Mobil - 1996 Belgian harvest

3. Process

Setup

Malts were made by two different malting processes :

- A6. traditional malting
- (without inoculation of any spore suspension)
- D6. malting process according to the invention
- (inoculation of the steeped wheat during the first wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

Steeping

- the steeping was carried out in a 2 kg base with a total water (tap water) to air ratio of 1.5:1;

- use was made of 2 fermentors (Bioflo III, New Brunswick Scientific), in which a perforated plate was placed;
- temperature was only controlled during the wet stages; during the air rest stages the system was allowed to reach room temperature (± 20 °C);
- during the whole steeping period the wheat was aerated (4 liter sterile air per minute);
- steeping was carried out by immersion using the following scheme:

	Temperature (°C)	**Duration (h)**
First wet stage	13	6:00
First air rest stage	20	16:00
Second wet stage	14	4:00
Second air rest stage	20	16:00
Third wet stage	16	2:00

<u>Addition of the microbial cultures</u>

- ± during the steeping, 1.10^4 activated spores per gram air dry wheat were inoculated to the water of the first wet stage (D6);

<u>Germination</u>

- as described in example 4

<u>Kilning</u>

- as described in example 1

4. Methods of analysis and results

These were as described in example 1 (4. methods of analysis and results)

	Traditional malting process (A6)	Malting process according to the invention (D6)
Moisture	5.5	5.4
Extract	83.6	85.5
Extract difference	1.0	0.6
Color	3.9	7.6
Wort turbidity	1.4	1.4
Postcoloration	5.8	11.5
Total protein content	14.0	14.8
Soluble protein content	4.9	9.7
Kolbach index	35.0	65.5
Viscosity	1.99	1.79
pH	6.02	5.63
Diastatic power	183	193
Whole grains	19.4	20.2
Friability	35	42
Homogeneity	79.4	78.7
Filtration volume	220	295
β-glucanase activity	10.9	16,640
Xylanase activity	16.85	1,620.1

Example 7

1. Preparation of the microbial cultures

Strain

- S46: *Rhizopus oryzae* ATCC 9363

Preparation of the spore suspension

- the strain was grown on PDA (Potato Dextrose Agar, Oxoid) for approximately 7 days at 28 °C;
- the spores were harvested by flooding the culture with sterile physiological saline (0.9% NaCl) and by rubbing the sporulated mycelium gently with a sterile spatula;
- the spore supension was washed once with sterile physiological saline (0.9% NaCl) by centrifugation (3500 rpm, Jouan C312, for 15 min) and resuspended in sterile physiological saline (0.9% NaCl);
- the spore density was determined microscopically using a Thoma counting chamber.

Activation of the spore suspension

- 5.10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid), pH = 4.0 and incubated in a shaking water bath during 5 hours (1) at 42 °C;

2. Cereal

- Sorghum(S14)

3. Process

Setup

Malts were made by two different malting processes :

- A7. traditional malting
- (without inoculation of any spore suspension)
- D7. malting process according to the invention
- (inoculation of the sorghum during the first wet

stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

Cleaning

- washing of the sorghum is performed by using 6 liter tap water per kilogram sorghum and by removing the excess water

Steeping

- the steeping was carried out in a 2 kg base with a total water (tap water) to air ratio of 1.5:1;
- use was made of 2 fermentors (Bioflo III, New Brunswick Scientific), in which a perforated plate was placed;
- temperature was only controlled during the wet stages; during the air rest stages the system was allowed to reach room temperature (± 20 °C);
- during the whole steeping period the barley was aerated (2 liter sterile air per minute);
- steeping was carried out by immersion using the following scheme:

	Temperature (°C)	Duration (h)
First wet stage	28	10:00
First air rest stage	20	4:00
Second wet stage	28	10:00
Second air rest stage	20	4:00
Third wet stage	28	10:00
Third air rest stage	20	4:00

Addition of the microbial cultures

- during the steeping, 1.10^4 activated spores (1) per gram air dry sorghum were inoculated to the water of the first wet stage (D7);

Germination

- germination of ± 460 g steeped sorghum was carried out in a cylindrical container with perforated lids at a temperature of 28 °C during 4 days;
- air was supplied by natural diffusion;
- the containers were slowly rotated on an electronically controlled roller system (Cellroll ®, Tecnorama); i.e. every two hours the containers were rolled for 15 min at 1 rpm.

Kilning

- as described in example 1

4. Methods of analysis and results

These were as described in example 1 (4. methods of analysis and results).

	Traditional malting process (A7)	Malting process according to the invention (D7)
β-glucanase activity	98	991
Xylanase activity	524.72	413.48

Example 8 : Breadmaking

The performance of the wheat malts described in example 6 (A6 : traditional malting process; D6 : malting process according to the invention) were compared in a 100 g procedure described by Finney, K.F., An optimised straight-dough breadmaking method after 44 years, cereal Chemistry, 61, pp 20-27 (1984). In the recipe we used a commercial wheat flour, 6.0% sugar, 3.0% Crisco (Crisco, Procter and Gamble, Cincinatti, OH, USA), 1.5% salt and 2.5% yeast (Bruggeman, belgium). Malts were tested in a 0 to 0.25% concentration range and replaced an equal weight of flour.

Method of analysis and results

The bread specific volumes (i.e. the volume in cc per weight in g of bread) were determined using rapeseed displacement and the bread crumbs were evaluated. It was clearly observed that the malt according to the invention was a much more potent agent increasing the volume of the bread than malt obtained by the traditional malting process. At the same time we found no significant differences in the crumb structure of breads prepared with malt according to the invention and malt from the conventional process.

	Traditional malt (A6)	Malt according to the invention (D6)
Level of malt addition (%)	Specific volume of bread (cc/g)	Specific volume of bread (cc/g)
0.0	5.07	5.07
0.05	5.11	5.26
0.10	5.16	5.44
0.15	5.19	5.52
0.20	5.19	5.45
0.25	5.22	5.38

The present invention thus also includes the process for making bread showing an increased of the bread volume of 3% compared to bread of known malt.

CLAIMS

1. Process for the preparation of malted cereals, comprising one or more wetting stages at a temperature between 5 and 30 °C, preferably between 10 and 20 °C, until the material has a moisture content between 20 and 60% by weight, preferably between 38 and 47%, wherein after a germination period between 2 and 7 days, preferably between 3 to 6 days at a temperature between 10 and 30 °C, preferably between 14 and 18 °C, the moistened and germinated cereals are preferably kilned by increasing the temperature to values between 40 and 150 °C until the material has a moisture content between 2 and 15% by weight, and wherein one or more microbial cultures selected from the group comprising one or more bacteria and/or one or more fungi are added in one or more times either before or during or after the malting process of said cereals, characterised in that at least one of said microbial cultures is inoculated by means of activated spores, said activated spores being significantly more swollen than the dormant size, the size of the spores being increased by a factor preferably between 1.2 and 10 over the dormant size and/or having one or more germ tubes per spore.

2. Process according to claim 1 wherein the activation of the spores comprises at least one or a combination of the following treatments:

(a) cycles of wetting and/or drying,

(b) addition of nutritional supplies or addition of spore elements,

(c) exposure to temperatures changes, preferably within a range of 0 to 80 °C,

(d) exposure to changes in pH, preferably within a pH range of 2.0 to 8.0, more preferably between 3.0 and 6.0.

3. Process according to claim 1 or 2, for the preparation of malted barley, wherein the bacteria are selected from the group comprising Micrococcus spp., Streptococcus spp., Leuconostoc spp., Pediococcus spp. Lactococcus spp., Lactobacillus spp., Corynebacterium spp., Propionibacterium spp., Bifidobacterium spp., Streptomyces spp., Bacillus spp., Sporolactobacillus spp., Acetobacter spp., Agrobacterium spp., Alcaligenes spp., Pseudomonas spp., Gluconobacter spp., Enterobacter spp., Erwinia spp., Klebsiella spp., Proteus spp.

4. Process according to claim 1 or 2, for the preparation of malted barley wherein the fungi are selected from the group (genera as described by Ainsworth and Bisby's dictionary of the fungi, 8th edition, 1995, edited by DL Hawksworth, PM Kirk, BC Sutton, and DN Pegler (632 pp) Cab International) comprising Ascomycota preferentially Dothideales preferentially Mycosphaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericilla spp., Euroteum spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp.; Hypocreales preferentially Hypocreceae preferentially Hypocrea spp.; Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccharomycodaceae preferentially Hanseniaspora spp.; Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp.; Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Chlamydomucor spp., Mucor spp., Rhizopus spp., Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp., Mycoderma spp., Candida spp., Rhodotorula spp., Torulopsis spp., Geotrichum spp., Cladosporium spp., Trichoderma spp., Oidium spp., Alternaria spp., Helminthosporium spp., Aspergillus spp. as described by R.A. Samson ((1994) in Biotechnological handbooks, Volume 7 : Aspergillus, edited by Smith, J.E. (273 pp), Plenum Press), Penicillum spp.

5. Process according to claim 1 or 2 for the preparation of malted cereals other than malted barley wherein the bacteria are chosen from the group comprising Micrococcus spp., Streptococcus spp., Leuconostoc spp., Pediococcus spp., Lactococcus spp., Lactobacillus spp., Corynebacterium spp., Propionibacterium spp., Bifidobacterium spp., Streptomyces spp., Bacillus spp., Sporolactobacillus spp., Acetobacter spp., Agrobacterium spp., Alcaligenes spp., Pseudomonas spp., Gluconobacter spp., Enterobacter spp., Erwinia spp., Klebsiella spp., Proteus spp.

6. Process according to claim 1 or 2 for the preparation of malted cereals other than malted barley

wherein the fungi are chosen from the group comprising : Ascomycota preferentially Dothideales preferentially Mycophaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericilla spp., Euroteum spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp., Hypocreales preferentially Hypocreaceae preferentially Hypocrea spp., Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccaromycodaceae preferentially Hanseniaspora spp., Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp., Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Clamydomucor spp., Mucor spp., Rhizopus spp.; Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp., Mycoderma spp., Candida spp., Rhodotorula spp., Torulopsis spp., Geotrichum spp., Cladosporium spp., Trichoderma spp., Oidium spp., Alternaria spp., Helminthosporium spp., Aspergillus spp., Penicillium spp.

7. Process according to any of the preceding claims, wherein the moistening step is a steeping step and

total time of submersion in water during steeping step does not exceed 30 hours, preferentially takes 10 to 25 hours, or wherein the kilning includes more than two temperature steps and wherein the microbial culture comprises Rhizopus spp., Pseudomonas spp. and/or Aspergillus spp.

8. Process according to the claim 7, wherein the Rhizopus spp. is a Rhizopus oryzae such as a Rhizopus oryzae strain ATCC 9363.

9. Process according to the claim 7, wherein the Aspergillus spp. is an Aspergillus oryzae such as a Aspergillus oryzae strain ATCC 14156.

10. Process according to the claim 7, wherein the Pseudomonas spp. is Pseudomonas herbicola.

11. Process according to any of the preceding claims wherein said cereals are disinfected.

12. Malted cereal characterised by a β-glucanase activity increased by at least a factor 4 and/or a xylanase activity increased by at least a factor 4, compared to the conventional malting process of the corresponding cereal.

13. Malted barley, wherein the β-glucanase activity is higher than 700 U/kg. and/or the xylanase activity is higher than 250 U/kg.

14. Malted cereal according to claim 12 or 13 obtained by the process of any of the claims 1 to 12.

15. Malted cereal according to any of the claims 12 to 14, characterised in that they present an improved modification or an increased enzyme activity, e.g. an increased hydrolytic enzyme activity and/or a decreased level of toxins and/or increased microbial safety and/or acceptability.

16. Malted cereal according to any of the claims 12 to 15, which can have a significantly higher acrospire length.

17. Combination of cereals and at least one activated spore.

18. Use of the malted cereals according to any of the claims 12 to 15 obtainable by the process of any of the claims 1 to 12, for the preparation of beverages.

19. Use of the malted cereals according to any of the claims 12 to 15 obtainable by the process of any of the claims 1 to 12, in a detergent composition.

20. Use of the malted cereals according to any of the claims 12 to 15 obtainable by the process of any of the claims 1 to 12, as a bread additive.

21. Use of the malted cereals according to any of the claims 12 to 15 obtainable by the process of any of the claims 1 to 12, in animal feed compositions.

22. Use of the malted cereals according to any of the claims 12 to 15 obtainable by the process of any of the claims 1 to 12, in the bleaching technology.

23. Use of the malted cereals according to any of the claims 12 to 15 obtainable by the process of any of the claims 1 to 12, in the paper and pulp technology.

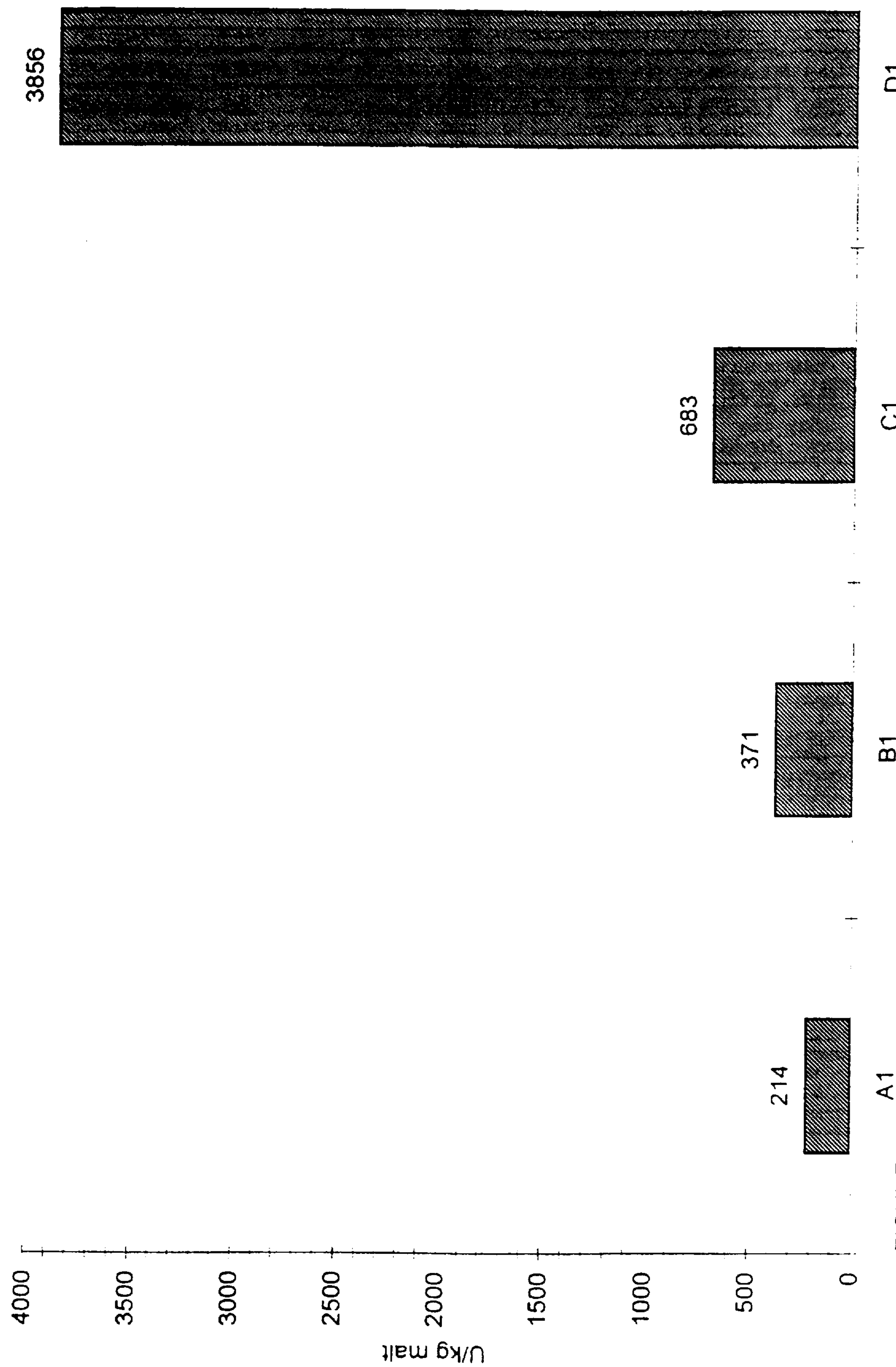

Glucanase activity
U/kg malt
4000
3500
3000
2500
2000
1500
1000
500
0
214
371
683
3856
A1
B1
C1
D1

FIGURE 1

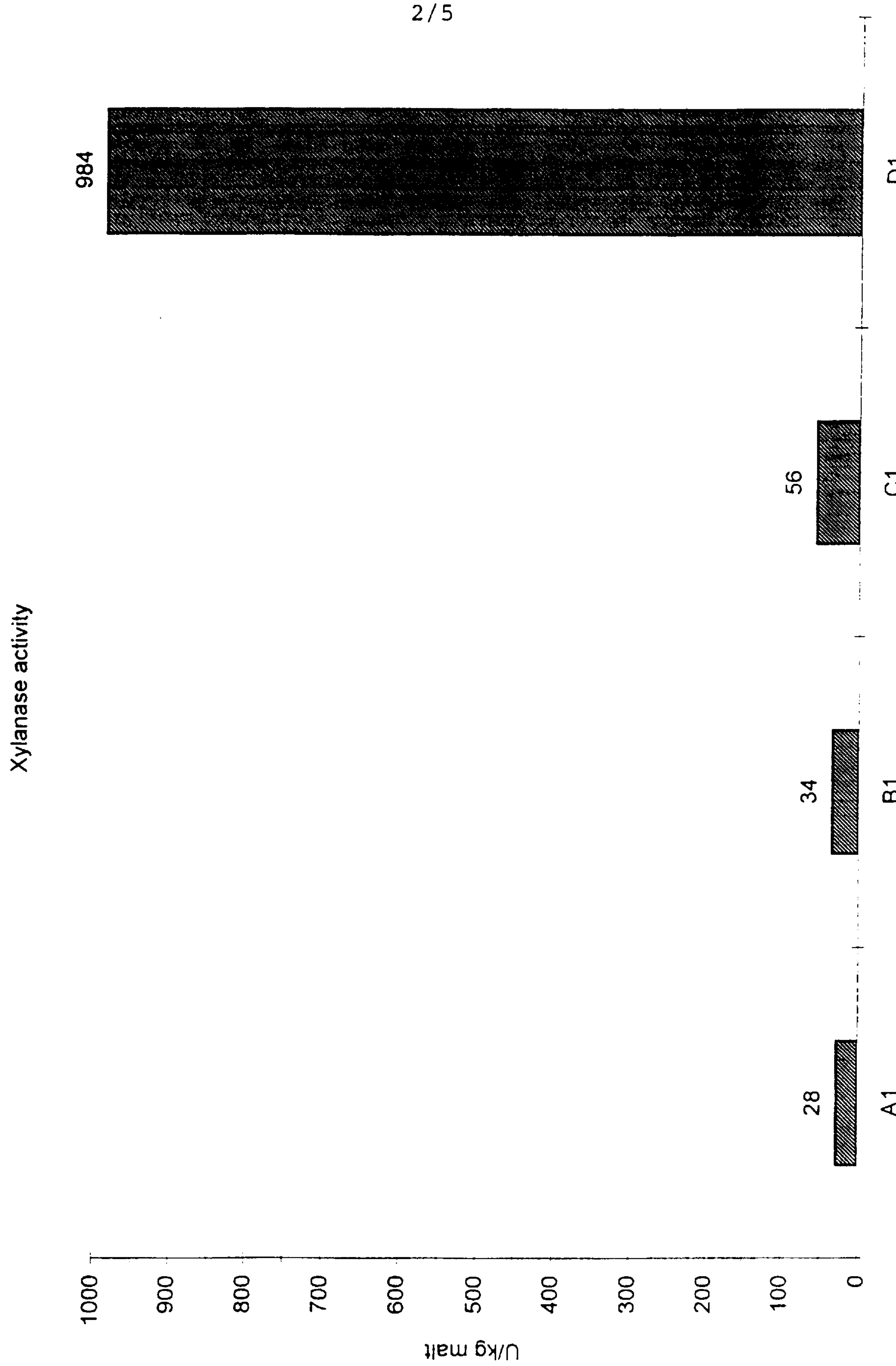

Xylanase activity
U/kg malt
1000
900
800
700
600
500
400
300
200
100
0
28
34
56
984
A1
B1
C1
D1

FIGURE 2

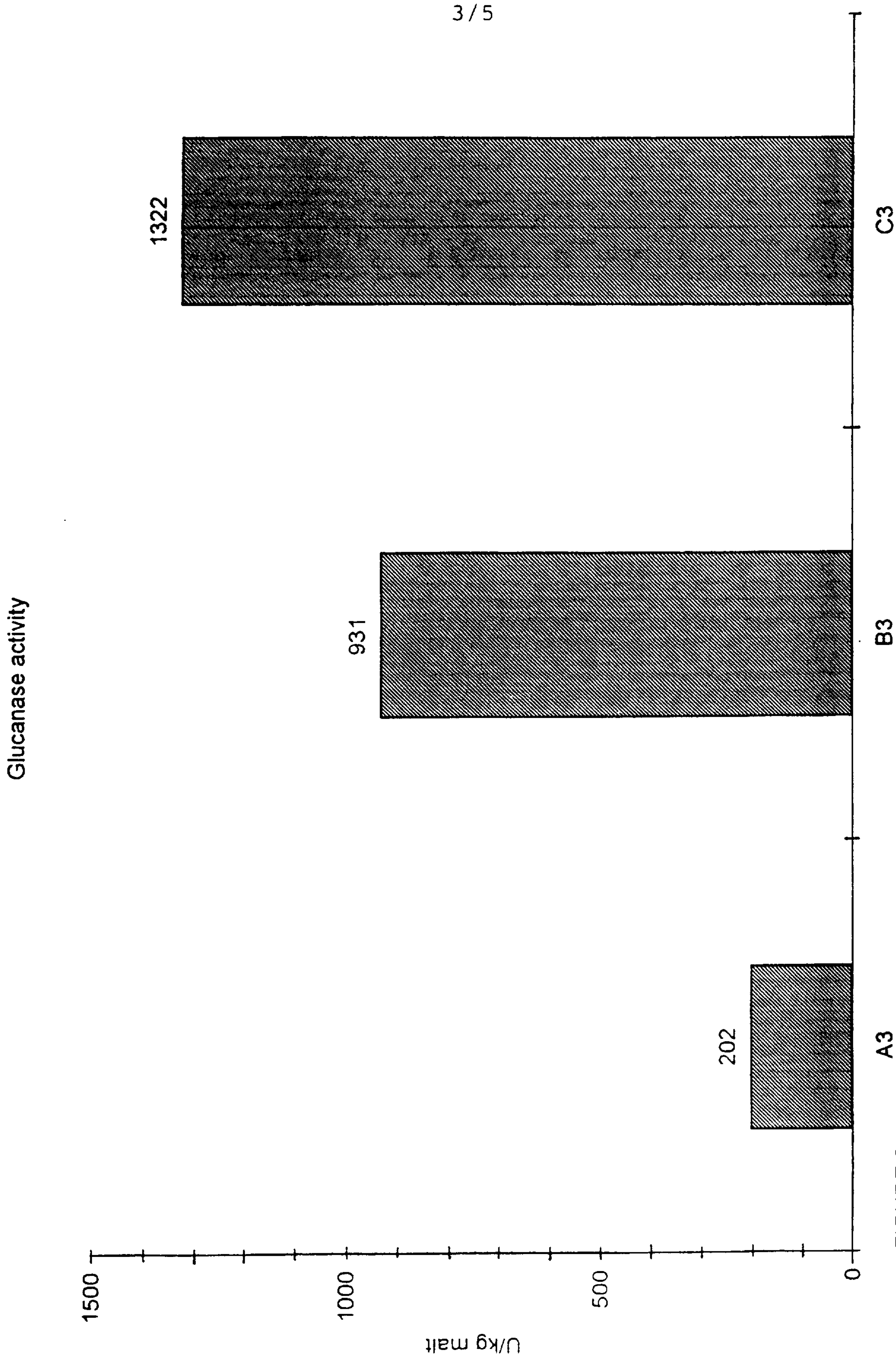

Glucanase activity
U/kg malt
1500
1000
500
0
1322
931
202
A3
B3
C3

FIGURE 3

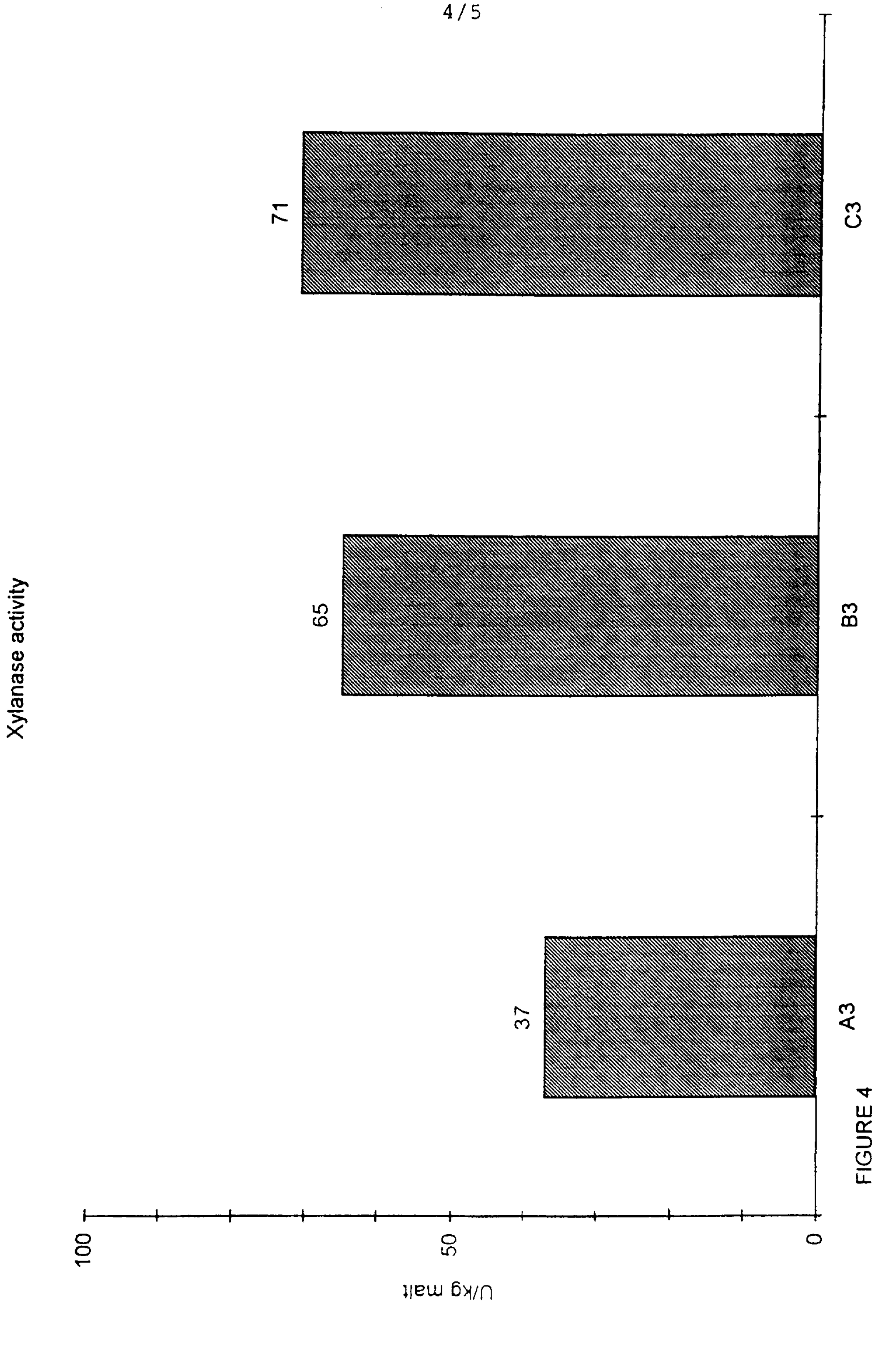
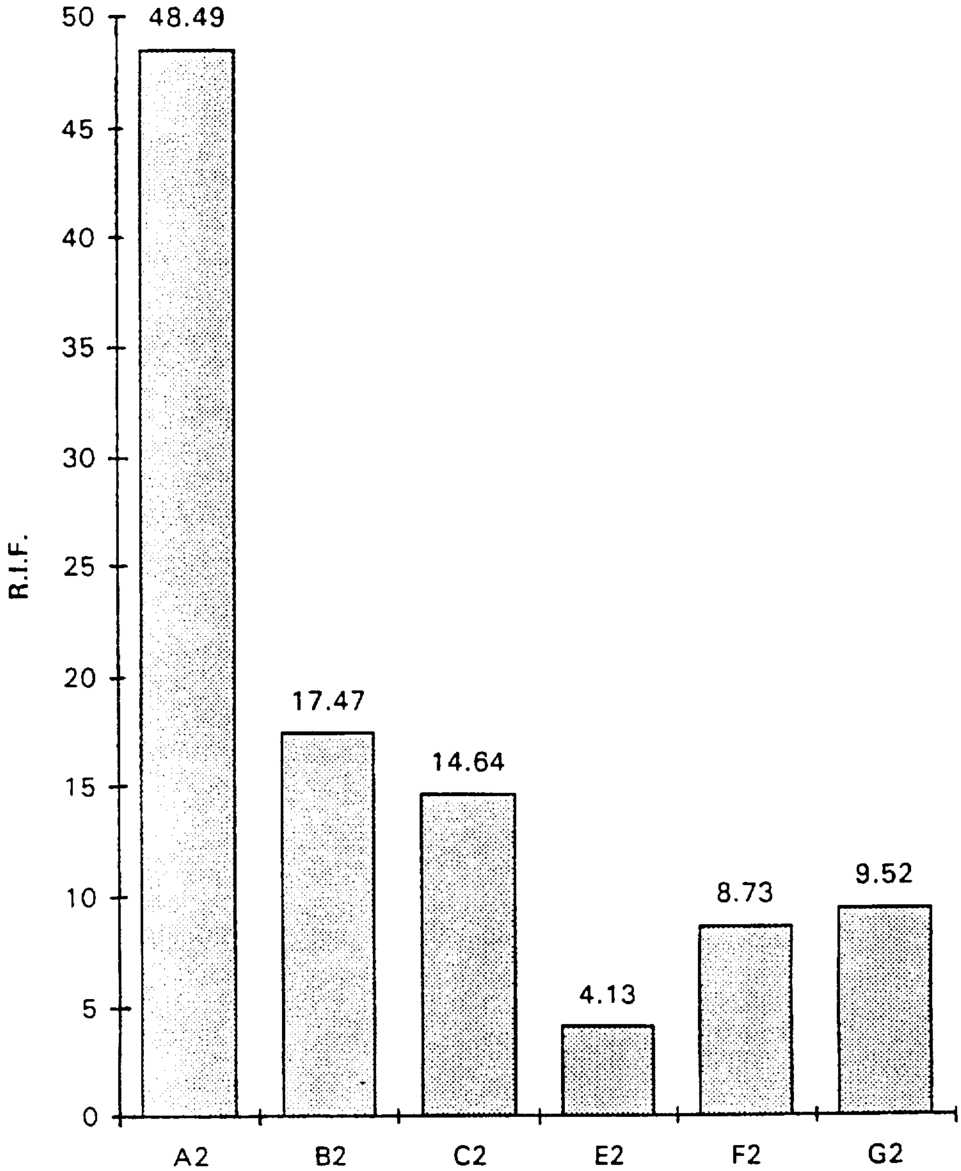


FIGURE 4



50
45
40
35
30
25
20
15
10
5
0
R.I.F.
48.49
17.47
14.64
4.13
8.73
9.52
A2
B2
C2
E2
F2
G2

FIGURE 5

INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/BE 97/00086

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12C1/00 C12N1/14 C12N1/20 C12C1/18

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)
IPC 6 C12C

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 practical, search terms used)

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	WO 94 16053 A (OY PANIFMOLABORATORIO-BRYGGERILABORATORIUM AB) 21 July 1994 see claims; table 6	1-3,5, 18-23
Y	GB 1 211 779 A (FORSCHUNGSINSTITUT FUER DIE GAERUNGSINDUSTRIE, ENZYMOLOGIE UND TECHNIS) 11 November 1970 see claims	1-3,5, 18-23

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
24 November 1997	02/12/1997

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/BE 97/00086

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	GYLLANG, H. ET AL.: "The influence of some fungi on malt quality." PROCEEDINGS, EUROPEAN BREWERY CONVENTION, vol. 16, 1977, pages 245-254, XP000671793 see page 247	1,2,4, 6-8, 18-23
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A	VAN WAESBERGHE, J.W.M.: "Microflora-management in industrial malting plants, outlook and opportunities." FERMENT, vol. 4, no. 5, 1991, pages 302-308, XP000671790	

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International Application No
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