Title: STABLE HAZE FOR BEVERAGES

A)

B)

Fig. 1

Abstract: The invention relates to a method for producing haze ingredients useful for production of hazy beverages. The invention further provides methods for producing hazy beverages. The methods involve treatment of starch with an α-amylase capable of hydrolyzing internal (1→4)-α-glucosidic linkages of starch, followed by separation of starch fragment particles according to size.
before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
Stable haze for beverages

Field of invention

The present invention relates to the field of haze ingredients useful for production of hazy beverages.

Background of invention

In certain beverages a hazy appearance is desirable. Frequently one or more "clouding agents" are added to beverages in order to produce a beverage with a hazy appearance. Frequently, clouding agents are emulsions of oils with as neutral a flavor as possible. Citrus oils are the most widely used, however due to the flavor, these have a limited use. Vegetable oil is also used, but less common due to poor resistance to oxidation. Vegetable gums have also been used as clouding agents, but quality has not always been reliable.

It has been suggested that beta-limit dextrins manufactured from dull waxy starch can be used in the manufacture of beverage clouding agents. Thus, US5,482,560 describes beta-limit dextrin produced by the action of a beta amylase on an aqueous slurry of dull waxy starch. It is described that these beta-limit dextrins can produce a solution having a solids content as high as 40% by weight at room temperature and thus the beta-limit dextrins are not useful as beverage clouding agents per se. The document further describes that emulsions of solutions of beta-limit dextrins with flavor oils can be used as clouding agents. The document describes the stability of aqueous emulsions and shows that absorbence decreases already after 35 days. Waxy starch is characterized by low levels of amylose, which are typically less than 10%, and frequently close to 0%.

Summary of invention

There is thus an unmet need for a haze ingredient, which is prepared from few ingredients and which is capable of providing a stable haze even upon long term storage. In particular, there is a need for a haze ingredient, which does not comprise oil, such as flavour oil. There is also a need for a haze ingredient with a neutral taste.
Interestingly, the present invention provides a haze ingredient that is prepared solely from starch and which provides a stable haze to a range of different beverages even upon long term storage. The haze ingredient has a neutral taste and is therefore widely applicable. The haze ingredient is prepared from starch and is thus particularly useful in beverages prepared from starch containing material, but can also be used in other kinds of beverages.

Thus, it is one aspect of the invention to provide methods of preparing a beverage with a haze, said method comprising the steps of

i) providing an aqueous composition comprising starch, wherein said starch comprises amylose and amylpectin;
ii) contacting said composition with an a-amylase capable of hydrolysing internal (1→4)-a-glucosidic linkages of starch thereby obtaining starch fragments contained in starch fragment particles;
iii) separating the starch fragment particles according to size;
iv) recovering starch fragment particles, wherein at least 40% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.03 μm to 500 μm, for example from 0.03 to 150 μm, more preferably in the range of 0.04 to 100 μm, such as from 0.04 to 30 μm, thereby obtaining a haze ingredient; and
v) providing a beverage
vi) mixing said beverage with said haze ingredient,

thereby preparing a beverage with a haze.

It is also an aspect of the invention to provide methods of preparing a haze ingredient, said method comprising the steps of

i) providing an aqueous composition comprising starch, wherein said starch comprises amylose and amylpectin;
ii) contacting said composition with an a-amylase capable of hydrolysing internal (1→4)-a-glucosidic linkages of starch thereby obtaining starch fragments contained in starch fragment particles;
iii) separating the starch fragments according to size;
iv) recovering starch fragment particles, wherein at least 40% of the recovered starch
fragment particles have a hydrodynamic diameter in the range from 0.03 μm to 500 μm, for example from 0.03 to 150 μm, more preferably in the range of 0.04 to 100 μm, such as from 0.04 μm to 30 μm,

thereby obtaining a haze ingredient.

It is furthermore an aspect of the invention to provide beverages and haze ingredients
prepared by said methods.

Description of Drawings

Figure 1 shows haze intensity over time of haze ingredient in a regular lager beer of
5% ABV stored for 55 days at 22°C (Panel A) or at 37°C (Panel B). Haze intensity was
measured at 90° angle using light at 650 nm.

Figure 2 shows the size distribution of hydrodynamic diameters of starch fragment
particles. Results were obtained by dynamic light scattering on a Malvern Mastersizer
3000. Scattering model: Mie. Result transformation type: volume. A) Size distribution of
starch fragment particles of haze ingredient prepared from barley starch using ethanol
precipitation for size separation. B) Size distribution of starch fragment particles of haze
ingredient prepared from barley starch using centrifugation for size separation. C) Size
distribution of starch fragment particles of haze ingredient prepared from maize starch
using ethanol precipitation for size separation. D) Size distribution of starch fragment
particles in haze ingredient prepared from potato starch using ethanol precipitation for
size separation.

Figure 3 shows haze intensity over time of haze ingredient in a regular lager beer of
5% ABV stored for 1 month at the indicated temperatures. Haze intensity was
measured at 90° angle using light at 650 nm. Panel A) shows the haze intensity of
haze ingredient prepared from barley starch. Panel B) shows the haze intensity of haze
ingredient prepared from wheat starch. Panel C) shows the haze intensity of haze
ingredient prepared from maize starch. Panel D) shows the haze intensity of haze
ingredient prepared from potato starch. Panel E) shows the haze intensity of haze
ingredient prepared from pea starch.
Figure 4 shows haze intensity of haze ingredient in lager beer stored at 22 °C for 100 days. The haze ingredient was prepared from starch of barley, wheat, maize, and pea using centrifugation for the size separation. Haze intensity was measured at 90° angle using light at 650 nm.

Figure 5 shows haze intensity over time of haze ingredient prepared from barley starch in apple juice and in a non-alcoholic malt based beverage stored for almost 60 days at the indicated temperatures. Haze intensity was measured at 90° angle using light at 650 nm.

Figure 6 shows the absolute molecular weight as a function of eluted volume (left hand axis and dotted line), as well as the relative amount as a function of eluted volume (right hand axis and normal line). A) shows the result for haze ingredient prepared from barley starch and B) the result for haze ingredient prepared from maize starch.

Figure 7 shows a proton spectrum obtained by NMR for a haze ingredient prepared from barley starch.

**Detailed description of the invention**

**Definitions**

The term "haze ingredient" as used herein refers to composition consisting of small particles, that when added to a transparent liquid, results in reduced transparency of said liquid.

The term "haze" as used herein refers to reduced transparency of a liquid. Thus, a beverage with a haze according to the invention, is a beverage, which is not completely transparent.

The term "internal glucosidic bond" as used herein refers to any glycosidic bond covalently linking two glucose residues of a starch molecule, wherein said glucose residues are positioned so that there are at least 3 glucose residues separating the internal glucosidic bond from an end of a chain of glucose residues of said starch.
The term "glycosidic linkage" as used herein refers to a covalent linkage between two glucose residues, and may be used interchangeably herein with the term "covalent glycosidic bond". The carbon atoms of the glucose units linked by a glycosidic linkage, may be specified by indicating the number of the carbon atoms. By way of example, then a glycosidic linkage connecting carbon 1 of one glucose unit with carbon 4 of another glucose unit may be referred to as a 1,4-glycosidic linkage. In starch, essentially all glycosidic linkages are α-glycosidic linkages.

The term "starch fragment" as used herein refers to a fragment of starch created by enzymatic cleavage of one or more internal glycosidic bonds. Starch fragments may have different sizes depending on the starting material, the degree of enzymatic cleavage and whether the starch fragments have been subjected to size separation.

Haze ingredient

The present invention relates to a haze ingredient, methods for producing the haze ingredient and to beverages comprising the haze ingredient.

The haze ingredient according to the present invention is preferably prepared from starch by enzymatic cleavage followed by size separation. Accordingly, the haze ingredient comprises or even consists of starch fragments. Such haze ingredient may also be referred to as dry haze ingredient. Upon contact with water, said starch fragments in general will form particles, which herein are designated "starch fragment particles". Said starch fragment particles preferably comprise water and starch fragments, and even more preferably the starch fragment particles consist of water and starch fragments. The haze ingredient may also comprise or consist of starch fragment particles. Such haze ingredient may also be referred to as wet haze ingredient.

A dry haze ingredient can be prepared from a wet haze ingredient by drying. Vice versa a wet haze ingredient can be prepared from a dry haze ingredient by mixing with water or another aqueous liquid.

Said starch fragments consist of linear or branched chains of glucose residues linked by glycosidic bonds. It is preferred that at least 50%, such as at least 60%, for example at least 70%, such as at least 80%, for example at least 90%, such as at least 95% of
the haze ingredient consists of starch fragments consisting of linear or branched chains of glucose residues linked by glycosidic bonds.

Thus, the haze ingredient preferably comprises or even consists of starch fragments, wherein the starch fragments when in an aqueous environment are contained in starch fragment particles. Thus, the haze ingredient may comprise or even consist of starch fragment particles, wherein the starch particle fragments preferably comprises or even consists of water and starch fragments, and it is preferred that at least 50%, such as at least 60%, for example at least 70%, such as at least 80%, for example at least 90%, such as at least 95% of the part of the starch fragment particles, which is not water consists of starch fragments consisting of linear or branched chains of glucose residues linked by glycosidic bonds. At least some of the starch fragment particles to be used with the present invention have a hydrodynamic diameter of in the range from 0.03 µm to 500 µm, preferably in the range from 0.03 to 150 µm, more preferably in the range of 0.04 µm to 100 µm, even more preferably from 0.04 µm to 30 µm, for example in the range from 0.04 to 1 µm.

Thus, it is preferred that at least 40% of the starch fragment particles of the haze ingredient have a hydrodynamic diameter of in the range from 0.03 µm to 500 µm, preferably in the range from 0.03 to 150 µm, more preferably in the range of 0.04 µm to 100 µm, even more preferably from 0.04 µm to 30 µm, yet more preferably in the range from 0.04 to 1 µm.

It is even more preferred that at least 50% of the starch fragment particles of the haze ingredient have a hydrodynamic diameter of in the range from 0.03 µm to 500 µm, preferably in the range from 0.03 to 150 µm, more preferably in the range of 0.04 µm to 100 µm, even more preferably from 0.04 µm to 30 µm, yet more preferably in the range from 0.04 to 1 µm.

In one embodiment at least 60%, such as at least 70%, for example at least 80% of the starch fragment particles of the haze ingredient have a hydrodynamic diameter of in the range from 0.03 µm to 500 µm, preferably in the range from 0.03 to 150 µm, more preferably in the range of 0.04 µm to 100 µm, even more preferably from 0.04 µm to 30 µm, yet more preferably in the range from 0.04 to 1 µm.
In another preferred embodiment of the invention at least 80% of the starch fragment particles of the haze ingredient have a hydrodynamic diameter of in the range from 0.03 to 500 μηι, preferably in the range of 0.03 to 150 μηι, more preferably in the range of 0.04 μηι to 100 μηι, yet more preferably in the range from 0.04 μηι to 40 μηι, even more preferably in the range from 0.04 to 30 μηι.

In another preferred embodiment of the invention at least 90% of the starch fragment particles of the haze ingredient have a hydrodynamic diameter of in the range from 0.03 to 500 μηι, preferably in the range of 0.03 to 150 μηι, more preferably in the range of 0.04 μηι to 100 μηι, yet more preferably in the range from 0.04 μηι to 40 μηι, even more preferably in the range from 0.04 to 30 μηι.

In another preferred embodiment of the invention at least 95% of the starch fragment particles of the haze ingredient have a hydrodynamic diameter of in the range from 0.03 to 500 μηι, preferably in the range of 0.03 to 150 μηι, more preferably in the range of 0.04 μηι to 100 μηι.

In particular it is preferred that at least 50% of the starch fragment particles of the haze ingredient have a hydrodynamic diameter in the range of 0.04 μηι to 100 μηι, more preferably from 0.04 to 40 μηι, even more preferably from 0.04 μηι to 30 μηι. Thus, for example at least 60% of the particles of the haze ingredient may have a hydrodynamic diameter in the range of 0.04 μηι to 100 μηι, more preferably from 0.04 μηι to 30 μηι.

For example at least 70% of the particles of the haze ingredient may have a hydrodynamic diameter in the range of 0.04 μηι to 100 μηι, more preferably from 0.04 μηι to 30 μηι. For example at least 80% of the particles of the haze ingredient may have a hydrodynamic diameter in the range of 0.04 μηι to 100 μηι, more preferably from 0.04 μηι to 30 μηι. Thus, for example at least 90% of the particles of the haze ingredient may have a hydrodynamic diameter in the range of 0.04 μηι to 100 μηι, more preferably from 0.04 μηι to 30 μηι. Thus, for example at least 95% of the particles of the haze ingredient may have a hydrodynamic diameter in the range of 0.04 μηι to 100 μηι.

In one embodiment of the invention it is preferred that at least 30% of the particles of the haze ingredient have a hydrodynamic diameter in the range of 0.04 μηι to 1 μηι. Thus, for example at least 40% of the particles of the haze ingredient may have a
hydrodynamic diameter in the range of 0.04 µηι to 1 µηι. For example at least 50% of the particles of the haze ingredient may have a hydrodynamic diameter in the range of 0.04 µηι to 1 µηι.

In one embodiment of the invention at least some of the particles of the haze ingredient have a hydrodynamic diameter in the range of 1 to 50 µηι. Thus, at least 1%, such as at least of the starch fragment particles may have a hydrodynamic diameter in the range of 1 to 50 µηι. For example at least 1%, such as at least 5% may have a hydrodynamic diameter in the range of 1 to 30 µηι.

According to the present invention the hydrodynamic diameter is preferably determined by dynamic light scattering using a Mastersizer 3000 instrument from Malvern Instruments Ltd., UK. More preferably the hydrodynamic diameter is determined by dynamic light scattering using a Mastersizer 3000 instrument from Malvern Instruments Ltd., UK using a Mie scattering model with instrument settings as described in Example 2.

It is also preferred that the starch fragments of the haze ingredient have a useful molecular weight.

Thus, in one embodiment the invention relates to a method for preparing a haze ingredient, said method comprising the steps of

i) providing an aqueous composition comprising starch, wherein said starch comprises amylose and amyllopectin, e.g. any of the starch described herein below;

ii) contacting said composition with an a-amylase capable of hydrolysing internal (1→4)-a- glucosidic linkages of starch, which e.g. may be any of the a-amylases describe herein below in the section "a-Amylase", thereby obtaining starch fragments contained in starch fragment particles,

iii) separating the starch fragments particles according to molecular weight of the starch fragments contained therein;

iv) recovering starch fragments particle containing starch fragments, wherein at least 75% of the starch fragments have a molecular weight in the range of 40 to 250,000 kDa,

thereby obtaining a haze ingredient. Said haze ingredient may be mixed with a beverage in order to produce a beverage with a haze.
In one embodiment of the invention at least 75% of the starch fragments have a molecular weight in the range of 50 to 250000 kDa, preferably in the range of 53 to 220000 kDa. This may in particular be the case in embodiments of the invention, wherein the starch is starch of barley.

In another embodiment of the invention at least 75% of the starch fragments have a molecular weight in the range of 40 to 100000 kDa, preferably in the range of 40 to 60000 kDa. This may in particular be the case in embodiments of the invention, wherein the starch is starch of maize.

Said starch fragments may have a molecular weight falling within the above-mentioned range and in addition having weight average molecular weight of in the range of 1000 to 10000 kDa, preferably in the range of 1500 to 5000 kDa, more preferably in the range of 1800 to 4000 kDa.

In one embodiment of the invention, the haze ingredient comprises starch fragments having a weight average molecular weight in the range of 2000 to 6000 kDa, preferably in the range of 3000 to 5000 kDa, even more preferably in the range of 3500 to 4600 kDa. This may in particular be the case in embodiments of the invention, wherein the starch is starch of barley.

In another embodiment of the invention, the haze ingredient comprises starch fragments having a weight average molecular weight in the range of 500 to 4000 kDa, preferably in the range of 1000 to 3000 kDa, even more preferably in the range of 1500 to 2500 kDa. This may in particular be the case in embodiments of the invention, wherein the starch is starch of maize.

In some embodiments of the invention, the haze ingredient comprises starch fragments having a number average molecular weight in the range of 200 to 700 kDa, preferably in the range of 300 to 600 kDa, even more preferably in the range of 350 to 550 kDa.

In particular, the haze ingredient may comprise or consist of starch fragment particles having above mentioned hydrodynamic diameter and comprising starch fragments, wherein
75% of all starch fragments in the haze ingredient a have a molecular weight in the range of 40 to 250000 kDa; and/or

b) the starch fragments have a weight average molecular weight in the range of 1000 to 10000 kDa, preferably in the range of 1500 to 5000 kDa, more preferably in the range of 1800 to 4000 kDa; and/or

c) the starch fragments have a number average molecular weight in the range of 200 to 700 kDa, preferably in the range of 300 to 600 kDa.

Molecular weight may be determined by any useful method, but is preferably determined using a system combining asymmetric flow field-flow fractionation, multi-angle laser light scattering, and refractive index detection set up to measurement of starch. In particular, the molecular weight may be determined as described in Example 6 herein below, on an AF4-MALLS instrument available from Wyatt Technology. Average molecular weight may be calculated as outlined in Example 6 herein below.

As described above the haze ingredient comprises or even consists of starch fragments or starch fragment particles, wherein the starch fragment particles preferably consists of starch fragments and water. Said starch fragment may be linear or branched. It is preferred that at least some of the starch fragments are branched.

In linear starch fragments the individual glucose units are linked by 1,4 - glycosidic linkages, whereas branching in starch is introduced by 1,6-glycosidic linkages. Thus, the ratio of 1,6-glycosidic linkages to 1,4-glycosidic linkages is a measure for the degree of branching of starch. Starch fragments having a high ratio of 1,6-glycosidic linkages to 1,4-glycosidic linkages are highly branched, and vice versa. In amyllopectin the ratio of 1,6-glycosidic linkages to 1,4-glycosidic linkages is typically 1 to in the range of 24 to 30.

It is preferred that the haze ingredient of the invention comprises starch fragments, wherein said starch fragments have an intermediate degree of branching, which preferably is lower than in typical amyllopectin. Thus, the degree of branching may be intermediate between the degree of branching of amylose (essentially no branching) and amyllopectin.
In particular it is preferred that the haze ingredient of the invention comprises starch fragments, wherein said starch fragments have a ratio of 1,6-glycosidic linkages to 1,4-glycosidic linkages of 1 to in the range of 10 to 20, preferably 1 to in the range of 10 to 16, more preferably 1 to in the range of 11 to 15, yet more preferably 1 to in the range of 12 to 14.

In one embodiment the invention relates to a method for preparing a haze ingredient, said method comprising the steps of

i) providing an aqueous composition comprising starch, wherein said starch comprises amylose and amylopectin, e.g. any of the starch described herein below;

ii) contacting said composition with an a-amylase capable of hydrolysing internal (1→4)-a -glucosidic linkages of starch, which e.g. may be any of the a-amylases described herein below in the section "a-amylase", thereby obtaining starch fragments contained in starch fragment particles,

iii) separating the starch fragments particles according to degree of branching of the starch fragments;

iv) recovering starch fragment particles containing starch fragments having a ratio of 1,6-glycosidic linkages to 1,4-glycosidic linkages of 1 to in the range of 10 to 20, preferably 1 to in the range of 10 to 16, more preferably 1 to in the range of 11 to 15, yet more preferably 1 to in the range of 12 to 14,

thereby obtaining a haze ingredient. Said haze ingredient may be mixed with a beverage in order to produce a beverage with a haze.

Thus, it is preferred that the starch fragments are not exclusively amylose fragments, but rather that said fragments comprise some degree of branching.

The ratio of 1,6-glycosidic linkages to 1,4-glycosidic linkages may be determined by any useful method, preferably using NMR. For example the ratio of 1,6-glycosidic linkages to 1,4-glycosidic linkages may be determined as described herein below in Example 7.

In general the haze ingredient does not comprise any significant amounts of oil, and in particular, the haze ingredient does not comprise any significant amount of citrus oil. Similarly, the haze ingredient also preferably does not comprise any significant amount of terpene oil. Thus, the haze ingredient preferably comprises at the most 1% oil, for
example the haze ingredient comprises at the most 1% citrus oil, for example the haze ingredient comprises at the most 1% terpene oil.

It is also preferred that the haze ingredient does not comprise any significant amount of starch sodium octenyl succinate. Thus, preferably the haze ingredient comprises at the most 1% starch sodium octenyl succinate, and more preferably the haze ingredient comprises no detectable starch sodium octenyl succinate.

**Method of preparing haze ingredient**

The invention relates to a haze ingredient, which in particular may be the haze ingredient described herein above in the section "Haze ingredient". The haze ingredient may preferably be prepared by a method comprising the steps of

i) providing an aqueous composition comprising starch;

ii) contacting said composition with an a-amylase capable of hydrolysing internal (1→4)-α-glucosidic linkages of starch thereby obtaining starch fragments contained in starch fragment particles,

iii) separating the starch fragments according to size;

iv) recovering starch fragments particles, wherein at least 40% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.03 µm to 500 µm, for example from 0.03 to 150 µm, more preferably in the range of 0.04 to 100 µm, such as from 0.04 µm to 30 µm, for example from 0.04 to 1 µm.

The composition comprising starch may comprise any of the starches described herein below in the section "Starch". The composition may comprise only one kind of starch, for example barley starch or the composition may comprise a mixture of different starches. The composition may consist of starch, however frequently, the composition consists of a plant product comprising starch. Said plant product is typically a fraction of a plant, which is enriched in starch content. The composition comprising starch is in general an aqueous solution or suspension comprising said starch. Thus the composition comprising starch may in one embodiment consist of water and starch. In another embodiment the composition may consist of water and fractions of one or more plants, which are enriched in starch. The composition comprising starch may in yet another embodiment consist of water and plant parts. Said plant may be any of the...
plants, which are useful sources for starch described herein below in the section "Starch".

The $\alpha$-amylase capable of hydrolysing internal \((1\rightarrow4)\)-a- glucosidic linkages of starch may be any of the a-amylases described herein below in the section "a-amylase".

Separating the starch fragments according to size may for example be performed as described herein below in the section "Size separation".

The composition comprising starch may in general be contacted with said $\alpha$-amylase in any manner allowing for activity of said a-amylase. In general the composition comprising starch is contacted with said $\alpha$-amylase in an aqueous solution or suspension. Thus, as described herein above the composition comprising starch may be an aqueous solution or suspension comprising starch, and in these embodiments the $\alpha$-amylase may be added directly to said solution or suspension. The time for incubation may differ depending on the amount of $\alpha$-amylase used and the temperature. The amount of enzyme, the time of incubation and the temperature of incubation is preferably selected so as to obtain a large number starch fragments with a hydrodynamic diameter in the range of 0.03 to 500 $\mu$m, preferably in the range of 0.03 to 150 $\mu$m, more preferably in the range of 0.04 $\mu$m to 100 $\mu$m, yet more preferably in the range from 0.04 $\mu$m to 40 $\mu$m, even more preferably in the range of 0.04 $\mu$m to 30 $\mu$m, such as in the range of 0.04 to 1 $\mu$m.

Frequently, the composition comprising starch is contacted with said $\alpha$-amylase for in the range of 30 minutes to 10 hours, for example for in the range of 45 minutes to 10 hours, such as for in the range of 45 minutes to 5 hours, such as in the range of 1 to 5 hours, for example for in the range of 1 to 3 hours. For example the composition comprising starch may be incubated with the $\alpha$-amylase for in the range of 1 to 3 hours at a temperature in the range of 20 to 80°, such as in the range of 20 to 70°C.

The temperature is selected in order to allow activity of said a-amylase. In one embodiment of the invention it is preferred that at least part of the incubation of the composition comprising starch with $\alpha$-amylase is performed at a temperature above the gelatinization temperature of the starch used. Gelatinization temperature is in general a range, and it is preferred that at least part of the incubation of the composition
comprising starch with α-amylase is performed at a temperature above the peak gelatinization temperature of the starch used, such as at least 1 °C, for example at least 2 °C above the the peak gelatinization temperature of the starch used. For example, the peak gelatinization temperature for barley starch may be in the range of 64 to 67 °C, such as in the range of 65 to 66 °C. Thus, in embodiments of the invention wherein the starch comprises or consists of barley starch, it is preferred that at least part of the incubation with α-amylase is performed at a temperature above 64 °C, such as above 65 °C, for example above 66 °C, such as above 67 °C, for example above 68 °C, such as above 69 °C. Similarly, for wheat starch the peak gelatinization temperature may be in the range of 58 to 62 °C. Thus, in embodiments of the invention wherein the starch comprises or consists of wheat starch, it is preferred that at least part of the incubation with α-amylase is performed at a temperature above 60 °C, such as above 61 °C, for example above 62 °C, such as above 63 °C. For rye starch the gelatinization temperature may be in the range of 57 to 70 °C. Thus, in embodiments of the invention wherein the starch comprises or consists of rye starch, it is preferred that at least part of the incubation with α-amylase is performed at a temperature above 65 °C, such as above 66 °C, for example above 67 °C, such as above 68 °C, for example above 69 °C, such as above 70 °C. Similarly, for oat starch the gelatinization temperature may be in the range of 53 to 59 °C. Thus, in embodiments of the invention wherein the starch comprises or consists of oat starch, it is preferred that at least part of the incubation with α-amylase is performed at a temperature above 56 °C, such as above 57 °C, for example above 58 °C, such as above 59 °C. Similarly, for potato starch the peak gelatinization temperature may be in the range of 64 to 66 °C. Thus, in embodiments of the invention wherein the starch comprises or consists of potato starch, it is preferred that at least part of the incubation with α-amylase is performed at a temperature above 66 °C, such as above 67 °C, for example above 68 °C. Similarly, for maize starch the peak gelatinization temperature may be in the range of 69 to 72 °C. Thus, in embodiments of the invention wherein the starch comprises or consists of maize starch, it is preferred that at least part of the incubation with α-amylase is performed at a temperature above 70 °C, such as above 71 °C, for example above 72 °C. Similarly, for rice starch the gelatinization temperature may be in the range of 68 to 78 °C. Thus, in embodiments of the invention wherein the starch comprises or consists of rice starch, it is preferred that at least part of the incubation with α-amylase is performed at a temperature above 74 °C, for example above 75 °C, such as above 76 °C, for example above 78 °C. Similarly, for pea starch the peak gelatinization temperature may be in the
range of 64 to 67°C. Thus, in embodiments of the invention wherein the starch comprises or consists of pea starch, it is preferred that at least part of the incubation with α-amylase is performed at a temperature above 65 °C, such as above 66°C, for example above 67 °C, such as above 68°C, for example above 69°C.

It is however also preferred that at least part of the incubation of the composition comprising starch with α-amylase is performed at a temperature at which α-amylase retains activity. Thus, it is preferred that at least part of the incubation of the composition comprising starch with α-amylase, and even more preferably that most of the incubation of the composition comprising starch with α-amylase is performed at a temperature below 90 °C, preferably below 80 °C.

The incubation of the composition comprising starch may thus be performed at a temperature in the range of 20 to 80 °C, such as a temperature in the range of 20 to 70 °C, for example a temperature in the range of 50 to 80 °C, such as a temperature in the range of 50 to 75 °C, for example a temperature in the range of 50 to 70 °C.

In one embodiment of the invention the temperature is varied so that the composition comprising starch is contacted with said α-amylase at a relatively low temperature and the temperature may then be increased gradually. Thus, for example the initial temperature may be in the range of 15 to 30°C. Then the temperature may for example be increased to a temperature in the range of 50 to 75°C. The temperature may be increased gradually for example by 0.1 to 5°C per minute. For example the composition comprising starch may be incubated with said α-amylase for in the range of 30 to 60 minutes at a temperature in the range of 50 to 75°C.

Incubation of the composition comprising starch with the α-amylase results in generation of a composition comprising starch fragments. The composition comprising starch fragments may be further treated as described below.

The incubation may be performed in any useful container. It is preferred that the container is container in which the temperature can be controlled. For example the container may be a mash kettle, however many other kinds of containers may be employed with the methods of the invention.
In one embodiment of the invention, the method of preparing a haze ingredient does not involve use of endo-a-1,6-glucanohydrolase. Thus, it is preferred that no endo-a-1,6-glucanohydrolase is added to the aqueous composition comprising starch. Said endo-a-1,6-glucanohydrolase may for example be pullulanase or isoamylase or amylo-1,6-glucosidase.

The methods of the invention preferably comprise a step of inactivating the a-amylase subsequent to incubation of the composition comprising starch with said a-amylase. Thus, it is preferred that the α-amylase is inactivated at a time when a large proportion of the starch fragment particles have a hydrodynamic diameter in the range from 0.03 μηι to 100 μηι, such as from 0.04 μηι to 30 μηι, for example from 0.04 to 1 μηι. The a-amylase may for example be inactivated by increasing the temperature. Thus, in certain embodiments of the invention the method comprises a step of heating the composition comprising starch and α-amylase to a temperature of at least 90°, preferably to at least 95 °C, such as to around 100 °C for at least 30 min, such as for in the range of 30 to 120 minutes, for example for in the range of 30 to 75 minutes.

Thus, in one embodiment the method of the invention may comprise the steps of

i) providing an aqueous composition comprising starch, such as any of the starch described herein below in the section "Starch";

ii) incubating said composition with an α-amylase capable of hydrolysing internal (1→4)-α-glucosidic linkages of starch (e.g. any of the α-amylases described herein below in the section "α-amylase") at a temperature allowing activity of said a-amylase, thereby obtaining starch fragments contained in starch fragment particles,

iii) inactivating said a-amylase, for example by incubation at a high temperature as described above; and

iv) separating the starch fragments particles according to size (for example as described herein below in the section "Size separation")

v) recovering starch fragments, wherein at least 40% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.03 μηι to 500 μηι, for example from 0.03 to 150 μηι, more preferably in the range of 0.04 to 100 μηι, such as from 0.04 μηι to 30 μηι, for example from 0.04 to 1 μηι.
In certain embodiments of the invention the method comprises a step of phase separation. This step is optional and may be omitted. The phase separation is generally performed after inactivation of the α-amylase at high temperature by cooling the composition containing starch fragments slightly and leaving the mixture without stirring for an extended amount of time. Thus, the phase separation may for example be obtained by incubation of the composition containing starch fragments at a temperature in the range of 70 to 90 °C, such as at a temperature in the range of 75 to 85 °C, for example at a temperature in the range of 78 to 82 °C for in the range of 10 to 60 hours, such as in the range of 10 to 48 hours, for example for in the range of 10 to 24 hours, such as in the range of 15 to 48 hours, such as in the range of 15 to 24 hours, for example for in the range of 18 to 22 hours. Said incubation is preferably done in the absence of movement of said composition, i.e. in the absence of stirring or shaking. After said incubation the composition comprising starch fragments will generally have separated into a top phase and a bottom phase. In general both of these phases comprise starch fragments and either the top phase or the bottom phase may be employed for the further procedure. Also a mixture of the phases may be employed or the phase separation step may also be omitted.

The methods of the invention may comprise an additional step of retrograding the starch fragments after incubation with α-amylase. Retrogradation is the process in which starch fragments realign after gelatinization. Retrogradation is in general obtained by extended incubation at a low temperature. It is especially preferred that the methods comprises a step of retrogradation in embodiments of the invention where incubation with α-amylase is performed at least partly at a temperature above the gelatinization temperature of the starch used.

Retrogradation may for example be obtained by incubation of the composition comprising starch fragments at a temperature in the range of 2 to 10 °C, for example in the range of 2 to 8 °C, such as in the range of 2 to 7 °C, for example in the range of 4 to 6 °C. The incubation at said temperature is preferably extended, and thus the incubation is in general for at least 10 hours, such as at least 15 hours, for example at least 18 hours, such as for in the range of 10 to 40 hours, for example for in the range of 15 to 40 hours, such as in the range of 15 to 30 hours, for example for in the range of 15 to 25 hours, such as for in the range of 18 to 22 hours.
Thus, in embodiment the methods of the invention comprises the steps of
i) providing an aqueous composition comprising starch, such as any of the
starch described herein below in the section "Starch";
ii) incubating said composition with an a-amylase capable of hydrolysing
internal (1\to 4)-a- glucosidic linkages of starch (e.g. any of the a-amylases
described herein below in the section "a-amylase") at a temperature above
the gelatinization temperature of said starch, wherein said temperature
allows activity of said a-amylase, thereby obtaining starch fragments
contained in starch fragment particles,

iii) optionally inactivating said a-amylase, for example by incubation at a high
temperature as described above; and

iv) retrograding said starch fragments by incubation at a low temperature as
described above; and

v) separating the starch fragments according to size (for example as described
herein below in the section "Size separation");

vi) recovering starch fragment particles, wherein at least 40% of the recovered
starch fragment particles have a hydrodynamic diameter in the range from
0.03 \( \mu \text{m} \) to 500 \( \mu \text{m} \), for example from 0.03 to 150 \( \mu \text{m} \), more preferably in the
range of 0.04 to 100 \( \mu \text{m} \), such as from 0.04 \( \mu \text{m} \) to 30 \( \mu \text{m} \), for example from
0.04 to 1 \( \mu \text{m} \).

Size Separation

The invention relates to a haze ingredient comprising starch fragments or starch
fragment particles. When in an aqueous environment the starch fragments will be
contained in starch fragment particles comprising or consisting of water and starch
fragments. Said starch fragment particles preferably have a hydrodynamic diameter in
the range of 0.03 \( \mu \text{m} \) to 500 \( \mu \text{m} \), and it is preferred that at least 40% of the recovered
starch fragment particles have a hydrodynamic diameter in the range from 0.03 to 150
\( \mu \text{m} \), more preferably in the range of 0.04 to 100 \( \mu \text{m} \), such as from 0.04 \( \mu \text{m} \) to 30 \( \mu \text{m} \), for example from 0.04 to 1 \( \mu \text{m} \). Therefore, methods of preparing the haze ingredient
frequently comprises a step of separating starch fragments according to size and then
recovering the starch with a hydrodynamic diameter in the range from 0.03 \( \mu \text{m} \) to 500
\( \mu \text{m} \), for example from 0.03 to 150 \( \mu \text{m} \), more preferably in the range of 0.04 to 100 \( \mu \text{m} \),
such as from 0.04 \( \mu \text{m} \) to 30 \( \mu \text{m} \), for example from 0.04 to 1 \( \mu \text{m} \).
The size separation may be performed by any suitable method known to the skilled person to be useful for separating particles with a hydrodynamic diameter in the range from 0.03 \( \mu \text{m} \) to 500 \( \mu \text{m} \), preferably in the range from 0.03 to 150 \( \mu \text{m} \), more preferably in the range of 0.04 to 100 \( \mu \text{m} \), such as from 0.04 \( \mu \text{m} \) to 30 \( \mu \text{m} \), for example from 0.04 to 1 \( \mu \text{m} \) from smaller and larger particles.

In one embodiment of the invention the size separation is performed with the aid of precipitation. The precipitation may be done using any useful precipitating agent. The method may contain only one precipitation step or it may contain two or more sequentially precipitation steps. It is however important that sufficient precipitations are undertaken to recover starch fragments of the desired size. Thus, the method may for example contain the steps of:

i) contacting the aqueous composition comprising starch fragments particles with a precipitating agent in an amount capable of precipitating starch fragments with a hydrodynamic diameter larger than than 500 \( \mu \text{m} \), preferably larger than 150 \( \mu \text{m} \), more preferably larger than 100 \( \mu \text{m} \), such as capable of precipitating at least part of starch fragments particles with a hydrodynamic diameter larger than 30 \( \mu \text{m} \), such as larger than 1 \( \mu \text{m} \); and

ii) recovering the supernatant; and

iii) contacting the supernatant with a precipitating agent in an amount capable of precipitating starch fragment particles with a hydrodynamic diameter larger than 0.03 \( \mu \text{m} \), such as larger than 0.04 \( \mu \text{m} \); and

iv) Recovering the precipitate, thereby recovering starch fragment particles, wherein at least 40\%, such as at least 50\% of the starch fragment particles have a hydrodynamic diameter in the range from 0.03 \( \mu \text{m} \) to 500 \( \mu \text{m} \), for example from 0.03 to 150 \( \mu \text{m} \), more preferably in the range of 0.04 to 100 \( \mu \text{m} \), such as from 0.04 \( \mu \text{m} \) to 30 \( \mu \text{m} \), for example from 0.04 to 1 \( \mu \text{m} \).

The precipitating agent may be any precipitating agent known to the skilled person to be useful for the precipitation of carbohydrates, such as starch. For example, the precipitating agent may be selected from the group alcohols, small ketones and cationic detergents.
Said small ketone may for example be acetone. Said cationic detergent may for example be compounds comprising an alkyl chain containing at least 10 carbons, and a quaternary ammonium ion. Non-limiting examples of cationic detergents useful as precipitating agents includes cetyltrimethylammonium hydroxide or hexadecyltrimethyl ammonium bromide.

It is however preferred that the precipitating agent is an alcohol. Said alcohol may for example be a $\text{C}_1$-alkyl-$\text{OH}$ or an $\text{C}_{56}$-aryl-$\text{OH}$. Thus, the alcohol may for example be selected from the group consisting of ethanol, isopropanol and phenol. Preferably the precipitating agent is ethanol.

Thus, in a preferred embodiment of the invention the size separation comprises at least one ethanol precipitation. Preferably, the size separation involves at least two ethanol precipitations, and thus the size separation may contain the steps of:

i) contacting the composition comprising starch fragments with an amount of $\text{C}_1$-$6$-alkyl-$\text{OH}$, preferably ethanol capable of precipitating starch fragments with a hydrodynamic diameter larger than 500 $\mu$m, preferably larger than 150 $\mu$m, more preferably larger than 100 $\mu$m, such as capable of precipitating at least part of starch fragments particles with a hydrodynamic diameter larger than 30 $\mu$m, such as larger than 1 $\mu$m; and

ii) recovering the supernatant; and

iii) contacting the supernatant with an amount of $\text{C}_1$-$6$-alkyl-$\text{OH}$, preferably ethanol capable of precipitating starch fragments with a hydrodynamic diameter larger than 0.03 $\mu$m, such as larger than 0.04 $\mu$m; and

iv) recovering the precipitate, thereby recovering starch fragment particles, wherein at least 40%, such as at least 50% of the starch fragment particles have a hydrodynamic diameter in the range from 0.03 $\mu$m to 500 $\mu$m, for example from 0.03 to 150 $\mu$m, more preferably in the range of 0.04 to 100 $\mu$m, such as from 0.04 $\mu$m to 30 $\mu$m, for example from 0.04 to 1 $\mu$m.

The amount of precipitating agent to be employed will depend on the temperature at which the precipitation is performed. It is important that a concentration of precipitating agent is selected, which results in precipitation of starch fragments of sizes as described above.
In particular the size separation may comprise the steps of

a) contacting an aqueous solution containing the starch fragments with a C₁₋₆-alkyl-OH, for example ethanol to a final concentration in the range of 25 to 35%, such as in the range of 27 to 29%

b) separating the precipitate from the supernatant

c) recovering the supernatant

d) contacting the supernatant with a C₇₋₁₋₆-alkyl-OH, for example ethanol to a final concentration in the range of 40 to 60%, such as in the range of 45 to 55%

e) separating the precipitate from the supernatant

f) recovering the precipitate,

thereby recovering starch fragments with a hydrodynamic diameter in the range from 0.01 μM to 100 μM, such as from 0.04 μM to 30 μM.

These concentrations are in particular relevant in embodiments of the invention, where the precipitation is performed at room temperature, such as at a temperature in the range of 20 to 30°C, for example in the range of 20 to 25°C. In embodiments of the invention wherein the precipitation is performed at lower temperatures, then in general less C₁₋₆-alkyl-OH, for example ethanol may be used.

Recovering the supernatant or the precipitate may for example be done by centrifugation followed by separation of the supernatant from the precipitate. Alternatively, the precipitate may be allowed to settle by simple gravitation followed by separation of the supernatant from the precipitate.

Recovering the precipitate may also comprise a step of drying the precipitate in order to remove all supernatant. In particular, the precipitate may be recovered and then subjected to drying, for example freeze-drying or spray-drying. After drying the haze ingredient will in general be a dry haze ingredient comprising mainly starch fragments.

The size separation may also be performed by chromatographic method and in particular using size exclusion chromatography. The size separation may also be performed using Asymmetric Flow-Field-Flow-Fractionation (AF4).
The size separation may also be accomplished by centrifugation. In particular, the size separation may comprise one or more centrifugation steps followed by recovery of supernatant. In this embodiment the composition comprising the starch fragments may be stored at a low temperature for an extended period of time.

Thus, the method for preparing the haze ingredient may comprise the steps of

i) providing an aqueous composition comprising starch, such as any of the starch described herein below in the section "Starch";

ii) incubating said composition with an a-amylase capable of hydrolysing internal (1→4)-α-glucosidic linkages of starch (e.g. any of the α-amylases described herein below in the section "α-amylase") at a temperature above the gelatinization temperature of said starch, wherein said temperature allows activity of said α-amylase, thereby obtaining starch fragments contained in starch fragment particles,

iii) optionally inactivating said α-amylase, for example by incubation at a high temperature as described above; and

iv) retrograding said starch fragments by incubation at a low temperature as described above; and

v) subjecting the retrograded composition comprising starch fragments to centrifugation; and

vi) recovering the supernatant; and

vii) optionally repeating steps v) and vi).

It is generally preferred that steps v) and vi) are performed more than once, such as twice, for example 3 times, such as 4 times, for example 5 times, such as in the range of 2 to 10 times, for example in the range of 3 to 7 times, such as in the range of 4 to 6 times.

The centrifugation may be performed for any suitable amount of time, such as for in the range of 5 to 60 minutes, such as in the range of 5 to 40 minutes, such as in the range of 10 to 30 minutes, for example for in the range of 10 to 20 minutes. In embodiments of the invention, where more than one centrifugation is performed, the time for each centrifugation may be the same or different and thus each centrifugation may individually be for in the range of 5 to 60 minutes, such as in the range of 5 to 40
minutes, such as in the range of 10 to 30 minutes, for example for in the range of 10 to 20 minutes.

The centrifugation may be performed at any speed, which is sufficiently high to settle particles larger than 500 μm, preferably larger than 150 μm, more preferably larger than 100 μm, such as capable of settle at least part of starch fragments particles with a hydrodynamic diameter larger than 30 μm, such as larger than 1 μm in the pellet.

Thus centrifugation may be performed for at in the range of 2000 to 10,000 xG, preferably in the range of 2500 to 7000 xG, more preferably in the range of 2800 to 5000 xG, such as in the range of 3000 to 4500 xG. In embodiments of the invention, where more than one centrifugation is performed, the speed for each centrifugation may be the same or different and thus each centrifugation may individually be for in the range of 2000 to 10,000 xG, preferably in the range of 2500 to 7000 xG, more preferably in the range of 2800 to 5000 xG, such as in the range of 3000 to 4500 xG.

It is also comprised within the invention that the speed of centrifugation may vary during the centrifugation. Thus for example the speed of centrifugation may increase during the centrifugation. This may be done step-wise or gradually.

Method of preparing a beverage

The invention also relates to methods for producing beverages, and in particular to methods of producing beverages with a haze. The invention also relates to beverages produced by the method.

The method for producing a beverage in general comprises the steps of

i) providing a haze ingredient according to the invention
ii) providing a beverage
iii) mixing said haze ingredient and said beverage.

The haze ingredient may be any of the haze ingredients described herein above, and said haze ingredient may be prepared by any of the methods for preparing a haze ingredient described herein above. The haze ingredient may be provided as a dry haze
An ingredient or as a wet haze ingredient. The beverage may be any beverages, for example any of the beverages described herein below in the section "Beverage".

In one embodiment the method of preparing a beverage with a haze thus comprises the steps of

i) providing an aqueous composition comprising starch, wherein said starch may be any of the starch described herein above in the section "Starch", and wherein said starch comprises amyllose and amylopectin;

ii) contacting said composition with an a-amylase capable of hydrolysing internal (1→4)-α-glucosidic linkages of starch (e.g. any of the a-amylases described herein below in the section "a-amylase") thereby obtaining starch fragments contained in starch fragment particles;

iii) separating the starch fragment particles according to size (for example as described herein above in the section "Size separation");

iv) recovering starch fragments, wherein at least 40% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.03 μm to 500 μm, for example from 0.03 to 150 μm, more preferably in the range of 0.04 to 100 μm, such as from 0.04 μm to 30 μm, for example from 0.04 to 1 μm, thereby obtaining a haze ingredient; and

v) providing a beverage, which for example may be any of the beverages described herein below in the section "Beverage";

vi) mixing said beverage with said haze ingredient,

thereby preparing a beverage with a haze.

In some embodiments of the invention, the starch fragments are recovered as a precipitate. This is in particular the case, when the seize separation is performed using precipitation with an alcohol.

When the haze ingredient is in the form of a precipitate, then it may be desirable to include a step of dispersing the haze ingredient between steps iv) and v) of the method.
Said additional step may comprise dispersing the haze ingredient in an aqueous solution. This may for example be obtained by incubating the haze ingredient in an aqueous solution (e.g. in water) at an elevated temperature, such as a temperature in the range of 90 to 100°C for in the range of 5 to 120 min., such as in the range of 10 to 60 min. e.g. for in the range of 15 to 30 min. Thus, the haze ingredient may be boiled in an aqueous solution (e.g. in water). The aqueous solution may then be mixed with said beverage.

**Beverage**

The beverage according to the present invention may be any beverage in which a haze is desirable. For many kinds of beverage a haze is desirable for aesthetic reasons.

In one embodiment of the invention the beverage is a plant derived beverage. A plant derived beverage may be any beverage prepared using plants, for example a plant derived beverage may be a beverage prepared by extraction of a plant or plant part in water or by squeezing a plant or plant part. Thus, the beverage may comprise or even consist of an extract or a juice of a plant or a plant part. The beverage may also be an extract or a juice of a plant or a plant part to which one or more additional ingredients have been added, for example flavouring compounds or preservatives.

Thus in one embodiment the beverage is a fruit juice, such as a juice of a citrus fruit or a pomaceous fruit. The citrus fruit may for example be selected from the group consisting of orange, lime, pomelo, lemon, mandarin, satsuma, grape fruit and kumquats. The pomaceous fruit may for example be selected from the group consisting of apple and pear.

In another embodiment the beverage is a fermented fruit juice. For example the beverage may be selected from the group consisting of fermented apple juice and fermented pear juice.

In yet another embodiment the beverage is an extract of a fruit, for example an extract of dried fruits or berries, for example the beverage may be an extract of a fruit selected from the group consisting of rosehip, sloe and crowberry.
The beverage may also be or comprise an extract of a plant for example a herbal extract. Non-limiting examples of herbal extracts includes an extract of green tea, black tea, rooibos, peppermint or hops. The extract of a plant may also be a flower extract. Non limiting examples of flower extracts includes hibiscus camomile, elderflower, lavender or linden flower. The extract of a plant may also be a fruit extract.

The flavour compound may be any useful flavour compound. The flavour compound may for example be selected from the group consisting of aromas, plant extracts, plant concentrates, plant parts and herbal infusions.

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

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

The beverage may also be any carbonated beverage, such as a carbonated soft drink. The carbonated soft drinks may be prepared from fruit extracts, fruit juices or fruit concentrates, however, the soft drinks may also be based on other flavours, such as ginger or tonic.

The carbonated beverage may also be an alcoholic drink.

In one embodiment of the invention the beverage is a cereal based beverage. In particular the beverage may be a malt based beverage. The malt based beverage may be any beverage prepared from malt. The malt will in general be barley malt, but in some embodiments of the invention the malt may also be malt of other cereal, for example wheat. In general, preparation of malt based beverages comprises a step of milling malt followed by an aqueous extraction, for example by mashing. The milled malt may be subjected to several rounds of aqueous extraction, for example to mashing and sparging. Preferably, the aqueous malt extract is wort, such as first wort, second wort, further worts or a mixture of one or more of the aforementioned.
It is also possible that the malt is mixed with one or more adjuncts before or during the aqueous extraction. The adjuncts may be any carbohydrate source other than malt, such as, but not limited to unmalted cereals e.g. barley, cereal syrups, cereal starch or grits. The cereal may be any cereal, for example any member of the Graminae plant family, cultivated primarily for their starch-containing seeds or kernels. Cereals include, but are not limited to barley (Hordeum), wheat (Triticum), rice (Oryza), maize (Zea), rye (Secale), oat (Avena), sorghum (Sorghum), and Triticale, a rye-wheat hybrid.

The wort may be further treated by a number of methods to arrive at the final beverage. The wort may for example be treated with one or more enzymes, such lipases, starch degrading enzymes (e.g. amylases, such as α-amylase, β-amylase and/or maltogenic α-amylase), glucanases [such as (1-4)- and/or (1-3,1-4)-glucanase], and/or xylanases (such as arabinoxylanase), and/or proteases, or enzyme mixtures comprising one or more of the aforementioned enzymes, e.g. Cereflo, Ultraflo, or Ondea Pro (Novozymes). In one embodiment of the invention the beverage has not been treated with protease, such as with proline-specific endo-proteases at any time during production of said beverage.

To obtain the cereal based beverage additional flavor compounds may also be added to the wort, e.g. hops. The wort may be heated or even boiled. Furthermore, the wort, such as the heated or boiled wort may also be subjected to fermentation, for example to fermentation by yeast. The cereal based beverage or the malt based beverage may also be carbonated.

In one preferred embodiment the beverage is beer, for example the beer may be a lager beer or an ale. Thus, the beer may for example be selected from the group consisting of altbier, Amber ale, Barley wine, Berliner weisse, Biere de Garde, Bitter, Blonde Ale, Bock, Brown ale, California Common, Cream Ale, Dortmunder Export, Doppelbock, Dunkel, Dunkelweizen, Eisbock, Fruit lambic, Golden Ale, Gose, Gueuze, Hefeweizen, Helles, India pale ale, Kolsch, Lambic, Light ale, Maibock, Malt liquor, Mild, Marzenbier, Old ale, Oud bruin, Pale ale, Pilsener, Porter, Red ale, Roggenbier, Saison, Scotch ale, Steam beer, Stout, Schwarzbier, lager, Witbier, Weissbier and Weizenbock.
The cereal based beverage may also be a non-alcoholic beverage. Thus, the malt based beverage may for example be a non-alcoholic beer or other kinds of non-alcoholic malt based beverages. Said non-alcoholic malt based beverages may also contain flavor compounds, such as fruit flavor compounds. Non-limiting examples of non-alcoholic malt based beverages includes Maltina, Moussy, or Holsten.

The beverage may also be any of the above-mentioned beverages to which one or more additional ingredients have been added, although in other embodiments the beverage is any of the aforementioned to which no further ingredients have been added except for the haze ingredient. Said additional ingredient may in one embodiment be an extract of a cereal and/or a malt extract, such as an extract made from non-malted cereals and malted barley. Examples of such an extract include an extract of oat, an extract of barley, a multigrain extract, an extract of wheat, an extract of non-malted oat and malted barley, an extract of non-malted barley and malted barley, a non-malted multigrain extract and malted barley, an extract of non-malted wheat and malted barley. Such extracts are for example Otex or Whetex available from Senson, Finland. Said additional ingredient may also be a polysaccharide, such as maltodextrin.

The beverages of the invention may be any of the aforementioned beverages containing the haze ingredient according to the invention.

In particular, the beverage may contain 0.5 to 10 g/L, such as 1 to 5 g/L, for example 1 to 3 g/L of dry haze ingredient. Thus, the beverage may be any of the aforementioned beverages containing 5 to 10 g/L, preferably 1 to 5 g/L, such as 1 to 4 g/L, for example 1 to 3 g/L of dry haze ingredient according to the invention.

In particular, it is preferred that the beverage contains sufficient haze ingredient for the beverage to appear hazy to the human eye. Thus, it is preferred that the haze intensity of the beverage is at least 4 EBC units, preferably at least 5 EBC units, for example at least 6 EBC units, when determined at 650 nm at 90° angle.

It is a hallmark of the haze ingredient according to the present invention that it can provide a stable haze, i.e. that a beverage containing the haze ingredient of the invention continues to be hazy even upon storage. In particular, beverages containing
in the range of 1 to 5 g/L, preferably in the range of 1 to 4 g/L of dry haze ingredient continues to be hazy even upon storage.

Thus, it is preferred that the haze intensity of the beverage determined as EBC units at 650 nm at 90° angle decreases at the most 20%, preferably at the most 10%, even more preferably at the most 5% upon storage at 22 °C for 50 days compared to the haze intensity after storage for 1 day.

It is also preferred that the haze intensity of the beverage determined as EBC units at 650 nm at 90° angle decreases at the most 20%, preferably at the most 10%, even more preferably at the most 5% upon storage at 37 °C for 50 days compared to the haze intensity after storage for 1 day.

It is especially preferred that in a beverage containing in the range of 1 to 5 g/L, preferably in the range of 1 to 4 g/L of dry haze ingredient according to the invention, then the haze intensity determined as EBC units at 650 nm at 90° angle decreases at the most 20%, preferably at the most 10%, even more preferably at the most 5% upon storage at 22 °C for 50 days compared to the haze intensity after storage for 1 day compared to the haze intensity after storage for 1 day.

It is also especially preferred that in a beverage containing in the range of 1 to 5 g/L, preferably in the range of 1 to 4 g/L of dry haze ingredient according to the invention, then the haze intensity determined as EBC units at 650 nm at 90° angle decreases at the most 20%, preferably at the most 10%, even more preferably at the most 5% upon storage at 37 °C for 50 days compared to the haze intensity after storage for 1 day.

In one embodiment it is preferred that the haze intensity of the beverage determined as EBC units at 650 nm at 90° angle is not altered by more than 20%, preferably not by more than 10% upon storage at 22 °C for 30 days compared to the starting haze intensity.

**Starch**

The invention relates to a haze ingredient and methods of preparing it from a composition comprising starch.
The composition comprising starch preferably comprises a high level of starch.

Frequently, the composition is an aqueous composition comprising starch dissolved or suspended in water. It is preferred that the composition apart from water comprises at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% starch. Thus, the composition comprising starch may consist of water and a non-water component and said non-water component preferably comprises at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% starch. In one embodiment of the invention the composition comprising starch consists of water and starch.

The starch is preferably homopolysaccharide composed only of glucoside units. In particular, the starch may comprise amylose and/or amylopectin. More preferably, the starch consists of amylose and/or amylopectin. Amylose is a homopolysaccharide composed only of glucoside units, wherein almost all are connected through a-D-1,4-glucosidic bonds. Amylopectin is a homopolysaccharide composed only of glucoside units, wherein in the range of 2 to 10% are connected through a-D-1,6-glucosidic bonds and the rest are connected through a-D-1,4-glucosidic bonds.

Depending on the source of the starch, the starch may have a different content of amylopectin and amylose. In one embodiment the starch comprises at least 10%, such as at least 15%, such as at least 20% amylose.

Thus, the starch may in one embodiment comprise in the range of 15 to 35%, preferably in the range of 20 to 35% amylose, for example in the range of 15 to 30%, more preferably in the range of 20 to 30% amylose. Aforementioned content of amylose may be advantageous to obtain a good yield of haze ingredient. For example the starch may comprise in the range of 20 to 30% amylose and in the range of 70 to 80% amylopectin. However, starch with a different composition of amylose and amylopectin can also be used with the present invention.

The starch may be starch of any useful source, in general the source will be a plant. Thus, the starch may be starch of a plant selected from the group consisting of barley, maize, wheat, rice, rye, oat, sorghum, pea, potato, cassava, sweet potato, millet, buckwheat, quinoa, amaranth, legumes and seaweed. In one embodiment the starch is
starch of a cereal, a tuber or a legume, preferably the starch is starch of a cereal or a tuber, more preferably of a cereal. The starch may also be a mixture of any of the aforementioned starches.

In one preferred embodiment of the invention the starch is barley starch. Barley starch in general comprises in the range of 20 to 30% amyllose and in the range of 70 to 80% amylopectin.

**a-Amylase**

The present invention relates to methods for preparing a haze ingredient using an α-amylase as well as to a haze ingredient prepared using these methods. The α-amylase to be used with the invention may be any α-amylase capable of hydrolysing internal (1→4)-α-glucosidic linkages. In particular it is preferred that the α-amylase to be used with the present invention is an enzyme capable of hydrolysing internal (1→4)-α-D-glucosidic linkages.

By the term "capable of hydrolyzing" as used herein is meant that the α-amylase is capable of catalysing hydrolysis.

By the term "internal glucosidic linkage" is meant a glucosidic linkage, which is positioned more than two glucose units away from the non-reducing end of a glucoside polymer, e.g. starch.

Thus, preferably the α-amylase to be used with the present invention is an enzyme capable of catalysing hydrolysis of (1→4)-α-D-glucosidic linkages, which are located more than two glucose-units away from the non-reducing ends of polysaccharides. In particular, the α-amylase may be an α-amylase capable of hydrolizing (1→4)-α-D-glucosidic linkages of cyclodextrins.

In addition to above mentioned activity, the α-amylase may also contain one or more additional activities, although this is not required. For example the α-amylase may also be a maltogenic amylase, and thus the α-amylase may also be capable of hydrolyzing (1→4)-α-D-glucosidic linkages in polysaccharides so as to remove successive α-
maltose residues from the non-reducing ends of polysaccharides, in particular from starch.

In one preferred embodiment of the invention, the α-amylase is an anti-staling amylase.

Examples of useful antistaling amylases include Novamyl available from Novozymes, Denmark or G4 or G+ enzymes from Dupont, Denmark.

One example of an α-amylase which is particularly useful with the present invention is Novamyl. The activity of Novamyl is for example described by Christophersen et al. (1998): "enzymatic characterization of Novamyl, a thermostable alpha-amylase".

Starch/Starke 50(1), 39-45.

The α-amylase may be an α-amylase from any useful organism, such as from Bacillus stearothermophilus.

Thus, the α-amylase may for example be an enzyme of SEQ ID NO: 1 or a functional homologue thereof sharing at least 70% sequence identity there with, for example at least 75%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95%, such as at least 98% sequence identity therewith. Said sequence identity is determined over the entire length of SEQ ID NO:1. Said functional homologue is an enzyme capable of hydrolyzing both internal (1→4)-α-D-glucosidic linkages in polysaccharides as well as hydrolyzing (1→4)-α-D-glucosidic linkages in polysaccharides so as to remove successive α-maltose residues from the non-reducing ends of polysaccharides.

The α-amylase may also just contain the active part of the enzyme of SEQ ID NO:1. Thus, amino acid 1 to 33 of SEQ ID NO:1 is a signal peptide. Thus, the α-amylase to be used with the invention may also be amino acid 34 to 719 of SEQ ID NO:1 or a functional homologue thereof sharing at least 70%, for example at least 75%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95%, such as at least 98% sequence identity with amino acid 34 to 719 of SEQ ID NO:1 over the entire length. Said functional homologue is an enzyme capable of hydrolyzing both internal (1→4)-α-D-glucosidic linkages in polysaccharides as well as hydrolyzing (1→4)-α-D-glucosidic linkages in polysaccharides so as to remove successive α-maltose residues from the non-reducing ends of polysaccharides.
In another embodiment of the invention the α-amylase is a glucan 1,4-α-maltotetraohydrolase, which also is capable of hydrolysing internal (1→4)-α-D-glcosidic linkages in amylase or amylopectin. Said glucan 1,4-α-maltotetraohydrolase may be derived from any useful organism, but in a preferred embodiment of the invention the glucan 1,4-α-maltotetraohydrolase is derived from *Pseudomonas saccharophila*. In particular it is preferred that the exo-amylase is *Pseudomonas saccharophila* maltotetraohydrolase of SEQ ID NO: 1 of international patent application WO201 0/1 33644 or a variant thereof sharing at least 65% identity therewith. Said variant preferably is capable of catalysing hydrolysis of (1→4)-α-D-glucosidic linkages in amylase or amylopectin to remove successive maltotetraose residues from the non-reducing chain ends and preferably shares at least 65%, more preferably at least 70%, even more preferably at least 75%, yet more preferably at least 80%, even more preferably at least 85%, yet more preferably 90% identity with SEQ ID NO: 1 of international patent application WO201 0/1 33644.

Preferred variants of *Pseudomonas saccharophila* maltotetraohydrolase to be used with the methods of the present invention are described in US patent applications US2005/037391, US2005/1 12237, US2006/008888, US2007/141693 and US2009/202675 as well as in international patent application WO201 0/1 33644, which are all incorporated by reference herein. Preferably, the variants of *Pseudomonas saccharophila* maltotetraohydrolase to be used with the methods of the present invention are any of the variants described in US patent applications US2005/037391, US2005/1 12237, US2006/008888, US2007/141693 and US2009/202675 as well as in the section “Polypeptide variants of SEQ ID NO:1 on international patent application WO201 0/1 33644.

**Examples**

The invention is further illustrated by the following examples, which however is not meant as being limiting for the invention.

**Example 1**

Pilot-scale preparation of haze ingredient from barley starch
25 kg barley starch (obtained from Altia, Finland) was dispersed in 250 L demineralised water in a mash kettle at a temperature of 24°C. 25 mL of a commercial preparation of amylase (PDN 17/6, obtained from Biocatalysts, UK) was added. Partial sequence information indicates that this enzyme is an enzyme of SEQ ID NO:1. The temperature was then increased at a rate of 1°C/minute to 66°C and kept at this level for 45 minutes. The temperature was then increased to 90 °C, again at a rate of 1°C/minute, and kept at this level for 60 minutes. The temperature was then increased to 100°C, again at a rate of 1°C/minute, and the mixture was boiled for 60 minutes. Throughout these steps, the mixture was stirred vigourously.

After the boiling step, the contents of the mash kettle were transferred hot to another tank, taking care not to let the temperature drop below 80 °C at any time during transfer. The mixture was then allowed to cool to 80 °C and left without stirring at this temperature for 20 hours. During this step, the mixture separated into an opaque top phase and a milky bottom phase. The top phase, about 70% of the total volume, was then transferred to storage containers using a pump and allowed to cool to 5°C. During this stage, the top phase turned milky white. The top phase was then left to retrograde at 5°C for at least 20 hours.

After retrogradation, starch fragment particles providing a stable haze were separated from small sugars, larger starch fragments, and undigested starch by step-wise precipitation with ethanol. Before precipitation, the retrograded top phase was attempered to 22 °C. In the first step, 96% ethanol was added to obtain a final concentration of 28% ABV. The mixture was then centrifuged twice, at 22 °C, for 15 minutes at 3000 xG. After each centrifugation, sediments were discarded. The concentration of ethanol in the supernatant was then brought to 50% ABV by addition of more 96% ethanol, and the preparation was left for at least 20 hours at 22 °C. After this period, the almost clear supernatant was decanted off and discarded. The remaining sediment was at first air-dried, then freeze-dried to remove remaining traces of ethanol, and finally ball-milled to a fine powder. The recovery of haze ingredient from several individual mashings, was 12.13%.

The purified, dried, and ball-milled haze ingredient was dispersed in water by boiling essentially as described in Example 3. The dispersed haze ingredient was added to a regular lager beer of 5% ABV to obtain a final concentration of 1 g/L, 2 g/L, 4 g/L or 6 g/L and stored either 22°C or 37 °C for more than 50 days, and the haze intensity was
Example 2
Size distribution of starch fragment particles

Haze ingredient was prepared from barley starch essentially as described in Example 1 i.e. the ingredient was prepared using ethanol precipitation for the size separation. Another haze ingredient was prepared from barley starch essentially as described in Example 4, i.e. the ingredient was prepared using centrifugation for the size separation. Two more haze ingredients were prepared from maize starch and potato starch essentially as described in Example 3, i.e. the ingredients were prepared using ethanol precipitation for the size separation. All haze ingredients were freeze-dried and finally ball-milled to fine powders.

The hydrodynamic diameters were measured by dynamic light scattering on a Mastersizer 3000 instrument from Malvern Instruments Ltd., UK using a Mie scattering model. Instrument settings were particle absorption index = 0.010, particle refractive index = 1.510, and dispersant refractive index = 1.330. On the day before analysis, each of the four haze ingredients obtained as described above were dispersed in water by boiling essentially as described in Example 3, and diluted in water to obtain a final concentration of 2 g/L. The samples were then stored at approx. 22°C until next day, when the hydrodynamic diameters of the starch fragment particles were measured by Particle Analytical, Horsholm, Denmark. The size distributions are shown in fig. 2A, 2B, 2C, and 2D. All four preparations contain starch fragment particles with hydrodynamic diameters in the range 0.03 µm to 1 µm. In addition the preparations also contain larger particles.

Example 3
Lab-scale preparation of haze ingredients from starches isolated from barley, wheat, maize, potato or pea
Barley starch was obtained from Altia, Finland. Starches from wheat, maize, potato or pea were obtained from Roquette, France. Each of these starches was mashed with amylase PDN 17/6 obtained from Biocatalysts, UK, essentially as described in example 1. However, the mashings were performed in a laboratory mashing apparatus, and the temperature during the first dwelling period was kept at 68 °C (barley, wheat, maize, and pea) or 65 °C (potato), instead of at 66 °C as described in example 1. The amounts of starch, water, and enzyme are listed in table 1.

<table>
<thead>
<tr>
<th>Starch type</th>
<th>Starch, g</th>
<th>Water, mL</th>
<th>Enzyme, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>240</td>
<td>2400</td>
<td>0.24</td>
</tr>
<tr>
<td>Wheat</td>
<td>240</td>
<td>2400</td>
<td>0.60</td>
</tr>
<tr>
<td>Maize</td>
<td>240</td>
<td>2400</td>
<td>0.42</td>
</tr>
<tr>
<td>Potato</td>
<td>40</td>
<td>400</td>
<td>0.28</td>
</tr>
<tr>
<td>Pea</td>
<td>240</td>
<td>2400</td>
<td>0.72</td>
</tr>
</tbody>
</table>

After phase separation at 80 °C, performed essentially as described in example 1, top phases and bottom phases were collected separately and allowed to retrograde at 8 °C for 16 hours. Haze ingredients were then purified by step-wise precipitations with ethanol at 28% ABV and 50% ABV, essentially as described in example 1, from the retrograded top phases obtained from barley, wheat, maize, potato and pea starch, and from retrograded bottom phase obtained from pea starch. The recoveries are listed in table 2.

<table>
<thead>
<tr>
<th>Starch type</th>
<th>Phase</th>
<th>Recovery of haze ingredient, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>Top</td>
<td>8.9</td>
</tr>
<tr>
<td>Wheat</td>
<td>Top</td>
<td>6.4</td>
</tr>
<tr>
<td>Maize</td>
<td>Top</td>
<td>8.4</td>
</tr>
<tr>
<td>Potato</td>
<td>Top</td>
<td>16.5</td>
</tr>
<tr>
<td>Pea</td>
<td>Top</td>
<td>2.2</td>
</tr>
<tr>
<td>Pea</td>
<td>Bottom</td>
<td>4.2</td>
</tr>
</tbody>
</table>
Haze intensity and haze stability of each of the purified, dried, and ball-milled ingredients were tested in a regular lager beer of 5% ABV. 2.5 g haze ingredient was dissolved in 50 mL water by boiling for 20 minutes, and 10-mL aliquots were then added to beer in 250-mL bottles. The final concentration of ingredient in beer was thus 2 g/L in all bottles. Samples containing haze ingredient prepared from barley, wheat, maize, or pea starch were stored at 5, 22, or 37 °C for 31 days, and haze was measured several times during storage. The haze intensity was measured on a LabScat instrument from Sigrist at both 90° and 25° angle using light at 650 nm. The outcome is provided as EBC UNITS. The haze intensity varied between ingredients, but all ingredients gave a stable haze at all storage temperatures. Results are shown in figures 3(A)-3(E).

Example 4
Lab-scale preparation of haze ingredients from starches isolated from barley, wheat, maize, or pea - alternative method

Starches from barley, wheat, maize, and pea were mashed with amylase PDN 17/6 essentially as described in example 3. After boiling, the mashes were immediately cooled to 8°C and kept at this temperature for 16 hours. The mashes were then attemperated to 22°C and centrifuged multiple times to remove larger starch fragments. Centrifugations were performed three times for 10 minutes at 3000 G, once for 20 minutes at 3000 G, and once for 20 minutes at 4500 G. After each centrifugation step, precipitated material was discarded. After these five centrifugations, 25-mL aliquots of the cloudy supernatants were added to regular lager beer (5% ABV) in 250-mL bottles. All samples were then stored at 22 °C for 100 days in order to test haze intensity and haze stability. The haze intensity was determined as described in Example 3. The haze intensity varied among the preparations, but all preparations gave a stable haze. Results are shown in figure 4.

Example 5
Stability of haze ingredient prepared from barley starch in non-alcoholic beverages

2.5 g haze ingredient, prepared from barley starch essentially as described in Example 1, was dissolved in 50 mL water by boiling for 20 minutes. 10-mL aliquots were then
added to either apple juice or a non-alcoholic malt beverage in 250-mL bottles. The final concentration of ingredient in the beverages was thus 2 g/L in all bottles. Samples were stored at 22 or 37 °C for almost 60 days, and haze was measured on a LabScat instrument from Sigrist, at both 90° and 25° angle at 650 nm several times during storage. The haze ingredient gave a stable haze in these beverages throughout the storage period. Results are shown in figure 5A and B.

Example 6

Haze ingredients were prepared from barley starch and maize starch as described in Example 3. The molecular weights of the starch fragments of the haze ingredient were then determined using asymmetric flow field-flow fractionation, multi angle laser light scattering, and refractive index detection set up to measurement of starch.

Molar mass can be obtained by MALLS, combined with detection by refractive index for concentration measurements, by applying the Rayleigh-Debye approximation:

\[
\frac{K^*c_i}{R(\theta)} = \frac{1}{M_1P(\theta)} + 2A_2c
\]

In this formula, \(R(9)\) is the excess Rayleigh scattering over and above that of the solvent due to a solute of concentration \(c_i\) for a slice \(i\) of the fractionated sample. \(K^*\) is an instrumental constant dependent on the wavelength and the refractive index increment \((dn/dc)\) of the solute in the particular solvent. \(M_1\) is the molar mass for the solute. \(P(\theta)\) is the scattering function that describes the angular dependence of the scattered light intensity. \(A_2\) is the second virial coefficient and captures non-ideality due to solvent-solute interactions.

The number average molecular weight \((M_n)\) and weight average molecular weight \((M_w)\) is derived by measuring \(R(9)\) at multiple angles for each slice \(i\) of the fractionated sample:

\[
M_n = \frac{\sum c_i}{\sum c_i/M_i}
\]
The concentration of each slice, \(c_i\), is conveniently measured by RI detection.

Prior to analysis the dried haze ingredients prepared from barley starch and maize starch as described in Example 3 were dispersed in the eluent (50mM \(\text{NaNO}_3\)) at concentrations 2.5 mg/ml and heated at 100°C for 30 min. and subsequently centrifuged at 7000 rpm for 5 min. 10-50 µl aliquots of the supernatants were analysed on an AF4-MALLS system from Wyatt Technology. In this system, AF4 flow control was maintained with a Wyatt Eclipse AF4 with Frit Inlet channel, which was in the thermost temperature control for Eclipse (kept at 60°C). The spacer for the Frit Inlet channel was the model 490W. For all experiments a regenerated cellulose membrane from Millipore was used (MWCO 10 kDa). Detection was accomplished with a Wyatt DAWN EOS 18-angle MALS (50°C) and an Optilab T-rEX refractive index detector (RI) (40°C).

The flow gradient was set as indicated in Table 3.

**Table 3**

<table>
<thead>
<tr>
<th>starting time (min)</th>
<th>end time(min)</th>
<th>Duration (min)</th>
<th>cross flow start (ml/min)</th>
<th>cross flow end (ml/min)</th>
<th>detector flow(ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>5</td>
<td>3.5</td>
<td>3.5</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>20</td>
<td>3.5</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>45</td>
<td>20</td>
<td>0.3</td>
<td>0.07</td>
<td>1</td>
</tr>
<tr>
<td>45</td>
<td>50</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>51</td>
<td>1</td>
<td>3.5</td>
<td>3.5</td>
<td>1</td>
</tr>
</tbody>
</table>

The MALLS detector was calibrated with toluene. Data were collected and processed with ASTRA software (V6.1.2.84). Degree of polynomial function was not used in the Zimm fit method for all the samples.

A refractive index increment (dn/dc) of 0.1454 ml/g and a second virial coefficient \(A_2\) of \(10^{-4}\) mol•ml/g² were used for all the starch samples in the processing of the data.
The results are provided in Table 4 below and in figure 6. Figure 6 shows the molecular weight as a function of eluted volume (left hand axis and dotted line), as well as the relative amount as a function of eluted volume (right hand axis and normal line). The molecular weight range of the samples (barley in figure 6A and maize in figure 6B) can be found from the analysis and the weight average and number average calculated. Table 4 shows the molecular weight of a fractionation containing at least 60% of the sample.

**Table 4**

<table>
<thead>
<tr>
<th></th>
<th>Lower limit molecular weight (kDa)</th>
<th>Upper limit molecular weight (kDa)</th>
<th>Weight average molecular weight, M_w (kDa)</th>
<th>Number average molecular weight, M_n (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley (top phase)</td>
<td>53</td>
<td>217100</td>
<td>4165</td>
<td>534</td>
</tr>
<tr>
<td>Maize (top phase)</td>
<td>45</td>
<td>51530</td>
<td>1819</td>
<td>384</td>
</tr>
</tbody>
</table>

**Example 7**

Haze ingredients were prepared from starches from barley, maize, potato, and pea as described in Example 3 and analysed by NMR. The samples were dispersed in D20. For each ingredient, a proton spectrum was obtained on a Bruker Avance 800 instrument, using 5 mm tubes and a run temperature of 37°C. The average ratios between reducing ends, α-1,4 glycosidic linkages, and α-1,6 glycosidic linkages in the haze ingredients were determined by integration of the NMR signals. Figure 7 shows the proton spectrum obtained for haze ingredient prepared from barley starch. Table 5 below summarises the average ratios between reducing ends, α-1,4 glycosidic linkages, and α-1,6 glycosidic linkages calculated for various haze ingredients.

**Table 5**

<table>
<thead>
<tr>
<th></th>
<th>α-1,4 : red.ends</th>
<th>α-1,6 : red.ends</th>
<th>α-1,4 : α-1,6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley (top phase)</td>
<td>35,6</td>
<td>2,8</td>
<td>12,7</td>
</tr>
<tr>
<td>Pea (bottom phase)</td>
<td>8,9</td>
<td>0,7</td>
<td>12,7</td>
</tr>
<tr>
<td>Potato (top phase)</td>
<td>31,5</td>
<td>2,4</td>
<td>13,1</td>
</tr>
<tr>
<td>Maize (top phase)</td>
<td>12,4</td>
<td>1,0</td>
<td>12,4</td>
</tr>
</tbody>
</table>
Claims

1. A method of preparing a beverage with a haze, said method comprising the steps of

i) providing an aqueous composition comprising starch, wherein said starch comprises amylase and amylopectin;

ii) contacting said composition with an α-amylase capable of hydrolysing internal (1→4)-α-glucosidic linkages of starch thereby obtaining starch fragments contained in starch fragment particles

iii) separating the starch fragment particles according to size;

iv) recovering starch fragment particles, wherein at least 40% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.03 µm to 500 µm, for example from 0.03 to 150 µm, more preferably in the range of 0.04 to 100 µm, such as from 0.04 µm to 30 µm, thereby obtaining a haze ingredient; and

v) providing a beverage

vi) mixing said beverage with said haze ingredient,

thereby preparing a beverage with a haze.

2. The method according to claim 1, wherein said beverage is selected from the group consisting of fruit juices and malt based beverages.

3. The method according to any one of the preceding claims, wherein the beverage is beer.

4. The method according to any one of the preceding claims, wherein the beverage is lager beer.

5. The method according to any one of the preceding claims, wherein the haze ingredient is added to said beverage in a concentration in the range of 0.5 to 10 g/L, preferably in the range of 1 to 5 g/L, more preferably in the range of 1 to 4 g/L, such as in the range of 1 to 3 g/L.

6. The method according to any one of the preceding claims, wherein the haze intensity of the beverage is at least 4 EBC unit at 650 nm at 90° angle.
7. The method according to any one of the preceding claims, wherein the haze intensity of the beverage determined as EBC units at 90° angle decreases at the most 20%, preferably at the most 10%, even more preferably at the most 5% upon storage at 22°C for 50 days compared to the haze intensity after storage for 1 day.

8. The method according to any one of the preceding claims, wherein the haze intensity in a beverage containing in the range of 1 to 4 g/L of the dry haze ingredient, then the haze intensity determined as EBC units at 650 nm at 90° angle decreases at the most 20%, preferably at the most 10%, even more preferably at the most 5% upon storage at 37°C for 50 days compared to the haze intensity after storage for 1 day.

9. The method according to any one of the preceding claims, wherein the method comprises an additional step between steps iv) and v) of dispersing the haze ingredient in an aqueous solution by boiling.

10. A method of preparing a haze ingredient, said method comprising the steps of
i) providing an aqueous composition comprising starch, wherein said starch comprises amylose and amyllopectin;
ii) contacting said composition with an a-amylase capable of hydrolysing internal (1→4)-a- glucosidic linkages of starch thereby obtaining starch fragments contained in starch fragment particles,
iii) separating the starch fragments according to size;
iv) recovering starch fragment particles, wherein at least 40% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.03 μm to 500 μm, for example from 0.03 to 150 μm, more preferably in the range of 0.04 to 100 μm, such as from 0.04 μm to 30 μm, thereby obtaining a haze ingredient.

11. The method according to any one claims 1 to 10, wherein step iv) consists of: recovering starch fragment particles having a hydrodynamic diameter in the range from 0.03 to 500 μm.

12. The method according to any one of claims 1 to 10, wherein step iv) consists of:
recovering starch fragment particles, wherein at least 40% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.03 to 1 \( \mu \text{m} \).

13. The method according to any one of claims 1 to 10, wherein step iv) consists of: recovering starch fragment particles, wherein at least 50% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.04 to 1 \( \mu \text{m} \).

14. The method according to any one of claims 1 to 10, wherein step iv) consists of: recovering starch fragment particles, wherein at least 80% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.04 to 30 \( \mu \text{m} \).

15. The method according to any one of claims 1 to 10, wherein step iv) consists of: recovering starch fragment particles, wherein at least 90% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.04 to 30 \( \mu \text{m} \).

16. The method according to any one of claims 1 to 10, wherein step iv) consists of: recovering starch fragment particles, wherein at least 95% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.04 to 100 \( \mu \text{m} \).

17. The method according to any one of claims 1 to 10, wherein step iv) consists of: recovering starch fragment particles, wherein at least 40% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.03 \( \mu \text{m} \) to 500 \( \mu \text{m} \), and wherein at least 1% of the recovered starch fragment particles have a hydrodynamic diameter in the range of 1 to 50 \( \mu \text{m} \), such as in the range of 1 to 30 \( \mu \text{m} \).

18. The method according to any one of the preceding claims, wherein at least 75% of the starch fragments have a molecular weight in the range of 40 to 250000 kDa, such as in the range of 50 to 250000 kDa, for example in the range of 53 to 220000 kDa.

19. The method according to any one of the preceding claims, wherein the starch fragments contained in the starch fragment particles have a weight average molecular weight in the range of 1000 to 10000 kDa, preferably in the range of 1500 to 5000 kDa, more preferably in the range of 1800 to 4000 kDa.
20. The method according to any one of the preceding claims, wherein the starch fragments contained in the starch fragment particles have a ratio of 1,6-glycosidic linkages to 1,4-glycosidic linkages of 1 to in the range of 10 to 20, preferably 1 to in the range of 10 to 16, more preferably 1 to in the range of 11 to 15, yet more preferably 1 to in the range of 12 to 14.

21. The method according to any one of the preceding claims, wherein the composition comprising starch consists of water and a non-water component and said non-water component comprises least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% starch.

22. The method according to any one of the preceding claims, wherein at least 90%, such as at least 95%, for example at least 98%, such as at least 99% of the content of the starch fragment particles consists of water and starch fragments.

23. The method according to any one of the preceding claims, wherein the starch comprises amylose and amylopectin.

24. The method according to any one of the preceding claims, wherein the starch comprises at least 10% amylose.

25. The method according to any one of the preceding claims, wherein the starch comprises in the range of 15 to 30% amylose.

26. The method according to any one of the preceding claims, wherein the starch comprises in the range of 20 to 30% amylose and in the range of 70 to 80% amylopectin.

27. The method according to any one of the preceding claims, wherein the starch is starch of a plant selected from the group consisting of barley, maize, wheat, rice, rye, oat, sorghum, pea, seaweed, cassava, sweet potato, millet, quinoa, amaranth, potato, buckwheat and legumes.

28. The method according to any one of the preceding claims, wherein the enzyme is selected from the group consisting of a-amylases capable of hydrolysing (1→4)-α-
glucosidic linkages, which are located more than two glucose-units away from the non-reducing ends of polysaccharides.

29. The method according to any one of the preceding claims, wherein said composition is contacted with said α-amylase for in the range of 1 to 10 hours, such as in the range of 1 to 5 hours.

30. The method according to any one of the preceding claims, wherein said composition is contacted with said α-amylase at a temperature in the range of 20 to 90°C.

31. The method according to any one of the preceding claims, wherein said method comprises a step of heating the composition comprising starch and α-amylase to a temperature of at least 95°C, such as to around 100°C for at least 30 min, such as for in the range of 30 to 120 minutes.

32. The method according to any one of the preceding claims, wherein said method comprises a step of retrograding the composition containing starch fragments at a temperature of in the range of 2 to 10°C for in the range of 10 to 40 hours.

33. The method according to any one of the preceding claims, wherein the step of separating the starch fragment particles according to size comprises at least one precipitation with C₁₋₆-alkyl-OH.

34. The method according any one of the preceding claims, wherein the step of separating the starch fragment particles according to size comprising the steps of:
   a) contacting an aqueous solution containing the starch fragment particles with C₁₋₆-alkyl-OH to a final concentration in the range of 25 to 35%, such as in the range of 27 to 29% at room temperature
   b) separating the precipitate from the supernatant
   c) recovering the supernatant
   d) contacting the supernatant with a Cᵥ₋₆-alkyl-OH to a final concentration in the range of 40 to 60%, such as in the range of 45 to 55% at room temperature
   e) separating the precipitate from the supernatant
   f) recovering the precipitate,
thereby recovering starch fragment particles with a hydrodynamic diameter in the range from 0.01 \( \mu m \) to 100 \( \mu m \), such as from 0.04 \( \mu m \) to 30 \( \mu m \).

35. The method according to any one of claims 33 to 34, wherein d-e-alkyl-OH is ethanol.

36. The method according to any one of the claims 1 to 32, wherein the step of separating the starch fragment particles according to size comprises at least one size separation by centrifugation.

37. The method according any one of the preceding claims 1 to 32, wherein the step of separating the starch fragment particles according to size comprising the steps of:
   a) centrifugation of an aqueous solution containing the starch fragments at in the range of 2500 to 5000 xG for in the range of 10 to 30 minutes,
   b) recovering the supernatant
   c) optionally repeating steps a) and b), wherein the xG and the time may be the same or different.

38. A haze ingredient prepared according to the method of any one of claims 9 to 37.

39. A haze ingredient consisting of starch fragment particles consisting of starch fragments and optionally water, wherein said starch fragment particles are characterised by one or more of the following characteristics:
   a) at least 40% of the recovered starch fragment particles have a hydrodynamic diameter in the range from 0.03 \( \mu m \) to 500 \( \mu m \), for example from 0.03 to 150 \( \mu m \), more preferably in the range of 0.04 to 100 \( \mu m \), such as from 0.04 \( \mu m \) to 30 \( \mu m \), for example from 0.04 to 1 \( \mu m \); and/or
   b) at least 75% of the starch fragments have a molecular weight in the range of 40 to 250000 kDa, such as in the range of 50 to 250000 kDa, for example in the range of 53 to 220000 kDa; and/or
   c) the starch fragments have a weight average molecular weight in the range of 1000 to 10000 kDa, preferably in the range of 1500 to 5000 kDa, more preferably in the range of 1800 to 4000 kDa; and/or
   d) the starch fragment have a ratio of 1,6-glycosidic linkages to 1,4-glycosidic linkages of 1 to in the range of 10 to 20, preferably 1 to in the
range of 10 to 16, more preferably 1 to in the range of 11 to 15, yet more preferably 1 to in the range of 12 to 14; and/or
d) the starch fragments have a number average molecular weight in the range of 200 to 700 kDa, preferably in the range of 300 to 600 kDa.

40. The haze ingredient according to claim 37, wherein the haze ingredient has all of characteristics b), c) and d).

41. The haze ingredient according to claim 37, wherein the haze ingredient has all of characteristics a), b), c) and d).

42. A beverage comprising a haze ingredient according to any one of claims 36 to 39.

43. The beverage according to claim 40, wherein the beverage further comprises at least one additional haze ingredient.

44. The beverage according to claim 41, wherein the additional haze ingredient is selected from the group consisting of an extract of a cereal, an extract of malt, a mixture of a extract of a cereal and an extract of malt, a polysaccharide and a flavour ingredient.
A) Haze ingredient from barley starch in beer

B) Haze ingredient from barley starch in beer

Fig. 1
Fig. 2
Fig. 2
Fig. 3
Fig. 3

C) Haze ingredient from maize starch (top phase)

D) Haze ingredient from potato starch (top phase)
E)

Haze ingredient from pea starch (bottom phase)

![Graph showing the haze at 90° angle in EBC-units over storage time in days at different temperatures: 5°C, 22°C, and 37°C.]

Fig. 3
Fig. 4
Fig. 5

A) Haze ingredient from barley starch in apple juice (2 g/L)

B) Haze ingredient from barley starch in non-alcoholic malt beverage (2 g/L)
A) Molar Mass vs. volume
Haze ingredient from barley (top phase)

B) Molar Mass vs. volume
Haze ingredient from maize (top phase)

Fig. 6
Reducing end: (1-6) : (1-4)
1 : 2.8 : 35.6

[rel]

α-1,4 glycosidic linkages

α-1,6 glycosidic linkages

[ppm]

Fig. 7
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12C11/11 A23L1/09 C12C12/00 C08B30/12 A23L2/62

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12C A23L C08B

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>X</td>
<td>EP 0 553 368 Al (NAT STARCH CHEM INVEST [US]) 4 August 1993 (1993-08-04) page 8, line 1 - line 2; claims 1-4; examples 1, 3-5 page 1, line 38 - line 39 page 5, line 43 - line 45</td>
<td>1-44</td>
</tr>
<tr>
<td>X</td>
<td>US 5 089 171 A (CHIU CHUNG-WAI [US]) 18 February 1992 (1992-02-18) col umn 2, line 7 - line 9; claims 1-4; examples 1, 3-5 col umn 7, line 13 - line 17</td>
<td>1-44</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

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A: document member of the same patent family

Date of the actual completion of the international search

9 April 2015

Date of mailing of the international search report

15/04/2015

Name and mailing address of the ISA:

European Patent Office, P.B. 5818 Patentlaan 2
N.L. - 2280 HV Rijswijk
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