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(54) **Title**: HYDROLYZED WHOLE GRAIN COMPOSITION

(57) **Abstract**: The present invention relates to a hydrolyzed whole grain composition comprising an alpha-amylase or fragments thereof, which alpha-amylase or fragments thereof show no hydrolytic activity towards dietary fibers when in the active state, and wherein the hydrolyzed whole grain composition has: (i) a glucose content of at least 0.25% by weight of the hydrolysed whole grain composition, on a dry matter basis; (ii) a maltose to glucose ratio below 144:1 by weight in the composition, (iii) a maltose to fructose ratio below 230:1 by weight in the composition or (iv) a maltose to glucose+fructose ratio below 144:1 by weight in the composition.

Hydrolyzed whole grain composition

Technical field of the invention

The present invention relates to hydrolyzed whole grain compositions. In particular the present invention relates to hydrolyzed whole grain compositions which have an optimized sugar profile and optimized organoleptic parameters such as taste and viscosity when used as an ingredient in food products.

Background of the invention

There is now extensive evidence emerging mainly from epidemiological studies that a daily intake of three servings of whole grain products, i.e. 48 g of whole grain, is positively associated with decreased risk of cardiovascular diseases, increased insulin sensitivity and decreased risk of type 2 diabetes onset, obesity (mainly visceral obesity) and digestive system cancers. These health benefits of the whole grains are reported to be due to the synergistic role of the dietary fibers and other components, such as vitamins, minerals and bioactive phytochemicals.

The regulatory authorities in Sweden, the US and the UK have already approved specific heart health claims based on the available scientific substantiation. Food products comprising dietary fibers are also growing in popularity with consumers, not just because whole grain consumption is now included in some national dietary recommendations but also because whole grain products are considered wholesome and natural. Recommendations for whole grain consumption have been set up by government authorities and expert groups to encourage consumers to eat whole grains. For instance, in the U.S.A, recommendations are to consume 45-80 g of whole grain per day. However, data provided by national dietary surveys in the United Kingdom, the U.S.A. and China show that whole grain consumption varies between 0 and 30 g whole grains per day.

The lack of whole grain products offered on the shelves and the poor organoleptic properties of the available whole grain products are generally identified as barriers for whole grain consumption and restrict the amount of whole grain to be added to food products, because, when increased amounts of whole grain are added the physical and organoleptic properties of the products might change dramatically.

Whole grains are also a recognised source of dietary fibers, phytonutrients, antioxidants, vitamins and minerals. According to the definition given by the American Association of Cereal Chemists (AACC), whole grains, and food made from whole grains, consist of the entire grain seed. The entire grain seed comprises the germ, the endosperm and the bran. It is usually referred to as the kernel.

Moreover, in recent years, consumers pay increased attention to the label of food products, and they expect manufactured food products to be as natural and healthy as possible. Therefore, it is desirable to develop ingredients for food and drink processing technologies and food and drink products that limit the use of non-natural food additives, even when such non-natural food additives have been fully cleared by health or food safety authorities.

Given the health benefits of whole grain cereal, it is desirable to provide a whole grain ingredient having as much intact dietary fibers as possible. To increase the whole grain content of a product or a serving, it is of course possible to increase the serving size. But this is not desirable as it results in a greater calorie intake. Another difficulty in just increasing the whole grain content of the product is that it usually impacts on physical properties such as the taste, texture and the overall appearance of the product (organoleptic parameters), as well as its processability.

The consumer is not willing to compromise on organoleptic properties, in order to increase his daily whole grain intake. Taste, texture and overall appearance are such organoleptic properties.

Obviously, industrial line efficiency is a mandatory requirement in the food industry. This includes handling and processing of raw materials, forming of the products, packaging and later storing, in warehouses, on the shelf or at home.

US 4,282,319 relates to a process for the preparation of hydrolyzed products from whole grain, and such derived products. The process includes an enzymatic treatment in an aqueous medium with a protease and an amylase. The obtained product may be added to different types of products. US 4,282,319 describes a complete degradation of the proteins present in the whole grain.

US 5,686,123 discloses a cereal suspension generated by treatment with both an alpha-amylase and a beta-amylase both specifically generating maltose units and have no glucanase effect.

Thus, it is an object of the present invention to provide a hydrolyzed whole grain composition that is rich in dietary fibers, and that provides an excellent consumption experience to the consumer when used in a food product, and that may be easily industrialised at a reasonable cost without compromising the organoleptic parameters.

Summary of the invention

Accordingly, in a first aspect the invention relates to a hydrolyzed whole grain composition comprising:

- an alpha-amylase or fragment thereof, which alpha-amylase or fragment thereof shows no hydrolytic activity towards dietary fibers when in the active state, and

  wherein the hydrolyzed whole grain composition has:

  a content of glucose of at least 0.25% by weight of the hydrolysed whole grain composition on a dry matter basis, such as at least 0.35%, e.g. at least 0.5%, and/or

  a maltose to glucose ratio below 144:1 by weight in the composition, such as a ratio below 120:1, such as a ratio below 100:1 e.g. a ratio below 50:1, such as a ratio below 30:1, e.g. a ratio below 20:1, such as a ratio below 1:10, or a maltose to fructose ratio below 230:1 by weight in the composition, such as below 144:1, such as below 120:1, such as below 100:1 e.g. below 50:1, such as below 30:1, such as below 20:1 or such as below 10:1, or

a maltose to glucose+fructose ratio below 144:1 by weight in the composition, such as below 120:1, such as below 100:1 e.g. below 50:1, such as below 30:1, such as below 20:1 or such as below 10:1.

Another aspect of the present invention relates to a process for preparing a hydrolyzed whole grain composition, said process comprising:

a) contacting a whole grain component with an enzyme composition in water, the enzyme composition comprising, at least one alpha-amylase, said enzyme composition showing no hydrolytic activity towards dietary fibers,
b) allowing the enzyme composition to react with the whole grain component, to provide a whole grain hydrolysate,
c) providing the hydrolyzed whole grain composition by inactivating said enzymes when said hydrolysate has reached a viscosity comprised between 50 and 5000 mPa.s measured at 65°C.

A third aspect of the invention relates to a food product comprising a hydrolyzed whole grain composition according to the invention.

Brief description of the drawings

Figure 1 shows a thin layer chromatography analysis of various enzymes put in contact with dietary fibres. The legend for the different tracks is the following:

A0: pure arabinoxylan spot (blank)
β0: pure beta-glucan spot (blank)
A: arabinoxylan spot after incubation with the enzyme noted below the track (BAN, Validase HT 425L and Alcalase AF 2.4L)
β: beta-glucan spot after incubation with the enzyme noted below the track (BAN, Validase HT 425L and Alcalase AF 2.4L)
E0: enzyme spot (blank)

Figure 2 shows size exclusion chromatography (SEC) of β-Glucan and arabinoxylan molecular weight profile without enzyme addition (plain line) and after incubation with Alcalase 2.4L (dotted line). A) Oat β-glucan; B) Wheat arabinoxylan.

Figure 3 shows size exclusion chromatography (SEC) of β-Glucan and arabinoxylan molecular weight profile without enzyme addition (plain line) and after incubation with Validase HT 425L (dotted line). A) Oat β-glucan; B) Wheat arabinoxylan.

Figure 4 shows size exclusion chromatography (SEC) of β-Glucan and arabinoxylan molecular weight profiles without enzyme addition (plain line) and after incubation with MATS L (dotted line) . A) Oat β-glucan; B) Wheat arabinoxylan.

Detailed description of the invention

The inventors of the present invention have surprisingly found that by treating the whole grain component with an alpha-amylase and optionally with a protease the whole grain will become less viscous and the following mixing into a food product may be easier. This results in the possibility to increase the amount of whole grains in the product. Furthermore, the alpha-amylase treatment also results in a reduced need for adding sweetener, such as sucrose, to the food product wherein the hydrolyzed whole grain composition according to the invention is used.

Thus in a first aspect the invention relates to a hydrolyzed whole grain composition comprising:

- an alpha-amylase or fragment thereof, which alpha-amylase or fragment thereof shows no hydrolytic activity towards dietary fibers when in the active state, and

wherein the hydrolyzed whole grain composition has:

a content of glucose of at least 0.25% by weight of the hydrolysed whole grain composition on a dry matter basis, such as at least 0.35%, e.g. at least 0.5%, and/or

a maltose to glucose ratio below 144:1 by weight in the composition, such as a ratio below 120:1, such as a ratio below 100:1 e.g. a ratio below 50:1, such as a ratio below 30:1, e.g. a ratio below 20:1, such as a ratio below 10:10, or

a maltose to fructose ratio below 230:1 by weight in the composition, such as below 144:1, such as below 120:1, such as below 100:1 e.g. below 50:1, such as below 30:1, such as below 20:1 or such as below 10:1, or

a maltose to glucose+fructose ratio below 144:1 by weight in the composition, such as below 120:1, such as below 100:1 e.g. below 50:1, such as below 30:1, such as below 20:1 or such as below 10:1.

Several advantages of having a food product comprising a hydrolyzed whole grain composition according to the invention may exist:

I. An increase in whole grain and fiber content may be provided in the final product, while the organoleptic parameters of the product are substantially not affected;
II. Dietary fibers from the whole grain may be preserved;
III. Greater sense of satiety substantially without affecting the organoleptic parameters of the product and slower digestion. Currently, there are limitations for enriching food products with whole grain due to non-flowable viscosity, grainy texture, and taste issues. However, the use of hydrolyzed whole grain according to the present invention in food products allow for providing the desired viscosity, a smooth texture, minimal flavour impact, and added nutritional health and wellness values;
IV. An additional advantage may be to improve the carbohydrate profile of the food product by replacing traditional externally supplied sweeteners such as glucose syrup, high fructose corn syrup, invert sugar, maltodextrin, sucrose, etc. with a more wholesome sweetener source.

A quality parameter of many food products and an important parameter in respect of the product processability is the viscosity of the hydrolysed whole grain composition. In the present context the term "viscosity" is a measurement of "thickness" or fluidability of a fluid. Thus, viscosity is a measure of the resistance of a fluid which is being deformed by either shear stress or tensile stress. If not indicated otherwise viscosity is given in mPa.s.

Viscosity may be measured using a Rapid Visco Analyser from Newport Scientific. The Rapid Visco Analyser measures the resistance of the product to the stirring action of a paddle. The viscosity is measured after 10 minutes stirring, at 65°C and 50 rpm.

The viscosity of the hydrolyzed whole grain composition according to the invention may vary. In an embodiment of the present invention, the viscosity measured at 65°C is in the range 1-4000 mPa.s, such as in the range 10-3000 mPa.s, such as in the range 10-1500 mPa.s, such as in the range 10-1000 mPa.s, such as in the range 10-500 mPa.s, such as in the range 2-500 mPa.s, or such as in the range 2-200 mPa.s. In an embodiment viscosity is measured at TS 50.

The whole grain component may be obtained from different sources. Examples of whole grain sources are semolina, cones, grits, flour and micronized grain (micronized flour). The whole grains may be grounded, preferably by dry milling. Such grounding may take place before or after the whole grain component being contacted with the enzyme composition according to the invention.

In an embodiment of the present invention the whole grain component may be heat treated to limit rancidity and microbial count.

Whole grains are cereals of monocotyledonous plants of the Poaceae family (grass family) cultivated for their edible, starchy grains. Examples of whole grain cereals include barley, rice, black rice, brown rice, wild rice, buckwheat, bulgur, corn, millet, oat, sorghum, spelt, triticale, rye, wheat, wheat berries, teff, canary grass, Job's tears and fonio. Plant species that do not belong to the grass family also produce starchy seeds or fruits that may be used in the same way as cereal grains, are called pseudo-cereals. Examples of pseudo-cereals include amaranth, buckwheat, tartar buckwheat and quinoa. When designating cereals, this will include both cereal and pseudo-cereals.

Thus, the whole grain component according to the invention may originate from a cereal or a pseudo-cereal. Thus, in an embodiment the hydrolyzed whole grain composition is obtained from a plant selected from the group consisting of barley, rice, brown rice, wild rice, black rice, buckwheat, bulgur, corn, millet, oat, sorghum, spelt, triticale, rye, wheat, wheat berries, teff, canary grass, Job's tears, fonio, amaranth, buckwheat, tartar buckwheat, quinoa, other variety of cereals and pseudo-cereals and mixtures thereof. In general the source of grain depends on the product type, since each grain will provide its own taste profile.

Whole grain components are components made from unrefined cereal grains. Whole grain components comprise the entire edible parts of a grain; i.e. the germ, the endosperm and the bran. Whole grain components may be provided in a variety of forms such as ground, flaked, cracked or other forms, as is commonly known in the milling industry.

In the present context the phrasing "a hydrolyzed whole grain composition" refers to enzymatically digested whole grain components or a whole grain component digested by using at least an alpha-amylase, which alpha-amylase shows no hydrolytic activity towards dietary fibers when in the active state. The hydrolyzed whole grain composition may be further digested by the use of a protease, which protease shows no hydrolytic activity towards dietary fibers when in the active state.

In the present context it is also to be understood that the phrase "a hydrolyzed whole grain composition" is also relating to enzymatic treatment of flour and subsequent reconstitution of the whole grain by blending flour, bran and germ. It is also to be understood that reconstitution may be done before the use in the final product or during mixing in a final product. Thus, reconstitution of whole grains after treatment of one or more of the individual parts of the whole grain also forms part of the present invention.

Prior to or after grinding of the whole grain, the whole grain component may be subjected to a hydrolytic treatment in order to breakdown the polysaccharide structure and optionally the protein structure of the whole grain component.

The hydrolyzed whole grain composition may be provided in the form of a liquid, a concentrate, a powder, a juice or a puree. If more than one type of enzymes is used it is to be understood that the enzymatic processing of the whole grains may be performed by sequential addition of the enzymes, or by providing an enzyme composition comprising more than one type of enzyme.

In the present context the phrase "an enzyme showing no hydrolytic activity towards dietary fibers when in the active state" should be understood as also encompassing the enzyme mixture from which the enzyme originates. For example, the proteases, amylases, glucose isomerase and amyloglucosidase described in the present context may be provided as an enzyme mixture before use which is not completely purified and thus, comprise enzymatic activity towards e.g. dietary fibers. However, the activity towards dietary fibers may also come from the specific enzyme if the enzyme is multi-functional. As used in here, the enzymes (or enzyme mixtures) are devoid of hydrolytic activity towards dietary fibers.

The term "no hydrolytic activity" or "devoid of hydrolytic activity towards dietary fibers" may encompass up to 5% degradation of the dietary fibers, such as up to 3%, such as up to 2% and such as up to 1% degradation. Such degradation may be unavoidable if high concentrations or extensive incubation times are used.

The term "In the active state" refers to the capability of the enzyme or enzyme mixture to perform hydrolytic activity, and is the state of the enzyme before it is inactivated. Inactivation may occur both by degradation and denaturation.

In general the weight percentages throughout the application are given as percentage by weight on a dry matter basis unless otherwise stated.

The hydrolyzed whole grain composition according to the invention may comprise a protease which shows no hydrolytic activity towards dietary fibers when in the active state. The advantage of adding a protease according to the invention is that the viscosity of the hydrolyzed whole grain may be further lowered, which may also result in a decrease in the viscosity of the final product. Thus, in an embodiment according to the invention the hydrolyzed whole grain composition comprises a protease or fragment thereof at a concentration of 0.0001 to 5% (w/w) by weight of the total whole grain content, such as 0.01-3%, such as 0.01-1%, such as 0.05-1%, such as 0.1-1%, such as 0.1-0.7%, or such as 0.1-0.5%. The optimal concentration of added proteases depends on several factors. As it has been found that the addition of protease during production of the hydrolyzed whole grain may result in a bitter off-taste, addition of protease may be considered as a tradeoff between lower viscosity and off-taste. In addition the amount of protease may also depend on the incubation time during production of the hydrolyzed whole grain. For example a lower concentration of protease may be used if the incubation time is increased.

Proteases are enzymes allowing the hydrolysis of proteins. They may be used to decrease the viscosity of the hydrolyzed whole grain composition. Alcalase 2.4L (EC 3.4.21.62), from Novozymes is an example of a suitable enzyme.

Depending on the incubation time and concentration of protease a certain amount of the proteins from the hydrolyzed whole grain composition may be hydrolyzed to amino acid and peptide fragments. Thus, in an embodiment 1-10% of the proteins from the whole grain composition is hydrolyzed, such as 2-8%, e.g. 3-6%, 10-99%, such as 30-99%, such as 40-99%, such as 50-99%, such as 60-99%, such as 70-99%, such as 80-99%, such as 90-99%, or such as 10-40%, 40-70%, and 60-99. Again protein degradation may result in a lowered viscosity and improved organoleptic parameters.

In the present context the phrase "hydrolyzed protein content" refers to the content of hydrolyzed protein from the whole grain composition unless otherwise defined. The protein may be degraded into larger or smaller peptide units or even into amino acid components. The person skilled in the art will know that during processing and storage small amount of degradation will take place which is not due to external enzymatic degradation.

In general it is to be understood that the enzymes used in the production of the hydrolyzed whole grain composition are different from the corresponding enzymes naturally present in the whole grain component.

It may be appropriate to evaluate the protein degradation on more specific proteins present in the whole grain composition. Thus, in an embodiment the degraded proteins are whole grain proteins, such as gluten proteins, globulins, albumins and glycoproteins.

Amylase (EC 3. 2. 1. 1) is an enzyme classified as a saccharidase: an enzyme that cleaves polysaccharides. It is mainly a constituent of pancreatic juice and saliva, needed for the breakdown of long-chain carbohydrates such as starch, into smaller units. Here, alpha-amylase is used to hydrolyse gelatinized starch in order to decrease the viscosity of the hydrolyzed whole grain composition. Validase HT 425L, Validase RA from Valley Research, Fungamyl from Novozymes and MATS from DSM are examples of alpha-amylases suitable for the present invention. Those enzymes show no activity towards the dietary fibers in the processing conditions used (duration, enzyme concentrations). On the contrary, e.g. BAN from Novozymes degrades dietary fibers besides starch into low molecular weight fibers or oligosaccharides, see also example 3.

In an embodiment of the present invention the enzymes show no activity towards the dietary fibers when the enzyme concentration is below 5% (w/w), such as below, 3% (w/w), e.g. below 1% (w/w), such as below 0.75% (w/w), e.g. below 0.5% (w/w).

Some alpha-amylases generate maltose units as the smallest carbohydrate entities, whereas others are also able to produce a fraction of glucose units. Thus, in an embodiment the alpha-amylase or fragments thereof is a mixed sugar producing alpha-amylase, including glucose producing activity, when in the active state. It has been found that some alpha-amylases both comprise glucose producing activity while having no hydrolytic activity towards dietary fibers when in the active state. By having an alpha-amylase which comprises glucose producing activity an increased sweetness may be obtained, since glucose has almost twice the sweetness of maltose. In an embodiment of the present invention a reduced amount of external sugar source needs to be added separately to a food product when a hydrolysed whole grain composition according to the present invention is used. When an alpha-amylase comprising glucose producing activity is used in the enzyme composition, it may become possible to dispense or at least reduce the use of other external sugar sources or non-sugar sweeteners.

In the present context the term "external sugar source" relates to sugars not originally present or originally generated in the hydrolysed whole grain composition. Examples of such external sugar source could be sucrose, glucose syrups, lactose, and artificial sweeteners.

Amyloglucosidase (EC 3.2.1.3) is an enzyme able to release glucose residues from starch, maltodextrins and maltose by hydrolysing glucose units from the non-reducing end of the polysaccharide chain. The sweetness of the preparation increases with the increasing concentration of released glucose. Thus, in an embodiment the food product further comprises an amyloglucosidase or fragments thereof. It may be advantageous to add an amyloglucosidase to the production of the hydrolyzed whole grain composition, since the sweetness of the preparation increases with the increasing concentration of released glucose. It may also be advantageous if the amyloglucosidase did not influence health properties of the whole grains, directly or indirectly. Thus, in an embodiment the amyloglucosidase shows no hydrolytic activity towards dietary fibers when in the active state. An interest of the invention, and particularly of the process for preparing food products comprising a hydrolyzed whole grain composition according to the invention, is that it allows reducing the sugar (e.g. sucrose) content of the food product when compared to products described in the prior art. When an amyloglucosidase is used in the enzyme composition, it may become possible to dispense with other external sugar sources e.g. the addition of sucrose.

However, as mentioned above certain alpha-amylases are able to generate glucose units, which may add enough sweetness to the product making the use of amyloglucosidases dispensable. Furthermore, application of amyloglucosidase also increases production costs of the hydrolyzed whole grain composition and, hence, it may be desirable to limit the use of amyloglucosidases. Thus, in yet an embodiment the hydrolyzed whole grain composition according to the invention does not comprise an amyloglucosidase such as an exogenic amyloglucosidase.

Glucose isomerase (D-glucose ketoisomerase) causes the isomerization of glucose to fructose. Thus, in an embodiment of the present invention the hydrolyzed whole grain composition further comprises a glucose isomerase or fragments thereof, which glucose isomerase or fragments thereof show no hydrolytic activity towards dietary fibers when in the active state. Glucose has 70-75% the sweetness of sucrose, whereas fructose is twice as sweet as sucrose. Thus, processes for the manufacture of fructose are of considerable value because the sweetness of the product may be significantly increased without the addition of an external sugar source (such as sucrose or artificial sweetening agents).

A number of specific enzymes or enzyme mixtures may be used for production of the hydrolyzed whole grain composition according to the invention. The requirement is that they show substantially no hydrolytic activity in the process conditions used towards dietary fibers. Thus, in an embodiment the alpha-amylase may be selected from Validase HT 425L and Validase RA from Valley Research, Fungamyl from Novozymes and MATS from DSM, the protease may be selected from the group consisting of Alcalase, iZyme B and iZyme G (Novozymes).

The concentration of the enzymes according to the invention in the hydrolyzed whole grain composition may influence the organoleptic parameters of a food product comprising said composition. In addition the concentration of enzymes may also be adjusted by changing parameters such as temperature and incubation time. Thus, in an embodiment the hydrolyzed whole grain composition comprises 0.0001 to 5% by weight of the total whole grain content in the composition of at least one of:

- an alpha-amylase or fragments thereof, which alpha-amylase or fragment thereof shows no hydrolytic activity towards dietary fibers when in the active state;

- an amyloglucosidase or fragment thereof, which amyloglucosidase shows no hydrolytic activity towards dietary fibers when in the active state; and
- a glucose isomerase or fragments thereof, which amyloglucosidase shows no hydrolytic activity towards dietary fibers when in the active state.

In yet an embodiment the hydrolyzed whole grain composition comprises 0.001 to 3% of the alpha-amylase by weight of the total whole grain content in the hydrolyzed whole grain composition, such as 0.01-3%, such as 0.01-0.1%, such as 0.01-0.5%, such as 0.01-0.1%, such as 0.03-0.1%, such as 0.04-0.1%. In yet an embodiment the hydrolyzed whole grain composition comprises 0.001 to 3% of the amyloglucosidase by weight of the total whole grain content in the hydrolyzed whole grain composition, such as 0.001-3%, such as 0.01-1%, such as 0.01-0.5%, such as 0.01-0.5%, such as 0.01-0.1%, such as 0.03-0.1%, such as 0.04-0.1%. In another further embodiment the hydrolyzed whole grain composition comprises 0.001 to 3% of the glucose isomerase by weight of the total whole grain content in the hydrolyzed whole grain composition, such as 0.001-3%, such as 0.01-1%, such as 0.01-0.5%, such as 0.01-0.5%, such as 0.01-0.1%, such as 0.03-0.1%, such as 0.04-0.1%.

Beta-amylases are enzymes which also break down saccharides, however beta-amylases mainly have maltose as the smallest generated carbohydrate entity. Thus, in an embodiment the hydrolyzed whole grain composition according to the invention does not comprise a beta-amylase, such as an exogenic beta-amylase. By avoiding beta-amylases a larger fraction of the starches will be hydrolyzed to glucose units since the alpha amylases do have to compete with the beta-amylases for substrates. Thus, an improved sugar profile may be obtained. This is in contrast to US 5,686,123 which discloses a cereal suspension generated by treatment with both an alpha-amylase and a beta-amylase.

In certain instances the action of the protease is not necessary, to provide a sufficient low viscosity. Thus, in an embodiment according to the invention, the hydrolyzed whole grain composition does not comprise the protease, such as an exogenic protease. As described earlier the addition of protease may generate a bitter off-taste which in certain instances is desirable to avoid. This is in contrast to US 4,282,319 which discloses a process including enzymatic treatment with a protease and an amylase.

In general the enzymes used according to the present invention for producing the hydrolyzed whole grain composition show no hydrolytic activity towards dietary fibers when in the active state. Thus, in a further embodiment the hydrolyzed whole grain composition has a substantial intact beta-glucan structure relative to the starting material. In yet a further embodiment the hydrolyzed whole composition has a substantial intact arabinoxylan structure relative to the starting material. By using the one or more enzymes according to the invention for the production of the hydrolyzed whole grain composition, a substantial intact beta-glucan and arabinoxylan structure may be maintained. The degree of degradation of the beta-glucan and arabinoxylan structures may be determined by Size-exclusion chromatography (SEC). This SEC technique has been described in more detail in "Determination of beta-Glucan Molecular Weight Using SEC with Calcofluor Detection in Cereal Extracts Lena Rimsten, Tove Stenberg, Roger Andersson, Annica Andersson, and Per Åman. Cereal Chem. 80(4):485–490", which is hereby incorporated by reference.

In the present context the phrase "substantial intact structure" is to be understood as for the most part the structure is intact. However, due to natural degradation in any natural product, part of a structure (such as beta-glucan structure or arabinoxylan structure) may be degraded although the degradation may not be due to added enzymes. Thus, "substantial intact structure" is to be understood that the structure is at least 95% intact, such as at least 97%, such as at least 98%, or such as at least 99% intact.

In the present context enzymes such as proteases, amylases, glucose isomerases and amyloglucosidases refer to enzymes which have been previously purified or partly purified. Such proteins/enzymes may be produced in bacteria, fungi or yeast, however they may also have plant origin. In general such produced enzymes will in the present context fall under the category "exogenic enzymes". Such enzymes may be added to a product during production to add a certain enzymatic effect to a substance. Similar, in the present context, when an enzyme is disclaimed from the present invention such disclaimer refers to exogenic enzymes. In the present context such enzymes e.g. provide enzymatic degradation of starch and proteins to decrease viscosity. In relation to the process of the invention it is to be understood that such enzymes may both be in solution or attached to a surface, such as immobilized enzymes. In the latter method the proteins may not form part of the final product.

As mentioned earlier, the action of the alpha-amylase results in a useful sugar profile which may affect taste and reduce the amount of external sugar or sweetener to be added to the final product.

In an embodiment of the present invention the hydrolysed whole grain composition has a glucose content of at least 0.25% by weight of the hydrolysed whole grain composition, on a dry matter basis, such as at least 0.35%, e.g. at least 0.5%.

Depending on the specific enzymes used the sugar profile of the final product may change. Thus, in an embodiment the hydrolyzed whole grain composition has a maltose to glucose ratio below 144:1, by weight in the composition, such as below 120:1, such as below 100:1 e.g. below 50:1, such as below 30:1, such as below 20:1 or such as below 10:1.

If the only starch processing enzyme used is a glucose generating alpha-amylase, a larger fraction of the end product will be in the form of glucose compared to the use of an alpha-amylase specifically generating maltose units. Since glucose has a higher sweetness than maltose, this may result in that the addition of a further sugar source (e.g. sucrose) can be dispensed. This advantage may be further pronounced if the ratio is lowered by the conversion of the maltose present in the hydrolyzed whole grain to glucose (one maltose unit is converted to two glucose units).

The maltose to glucose ratio may be further lowered if an amyloglucosidase is included in the enzyme composition since such enzymes also generates glucose units.

If the enzyme composition comprises a glucose isomerase a fraction of the glucose is changed to fructose which has an even higher sweetness than glucose. Thus, in an embodiment the hydrolyzed whole grain composition has a maltose to glucose+fructose ratio below 144:1 by weight in the composition, such as below

120:1, such as below 100:1 e.g. below 50:1, such as below 30:1, such as below 20:1 or such as below 10:1.

Furthermore, in an embodiment of the present invention the hydrolyzed whole grain composition may have a maltose to fructose ratio below 230:1 by weight in the composition, such as below 144:1, such as below 120:1, such as below 100:1 e.g. below 50:1, such as below 30:1, such as below 20:1 or such as below 10:1.

In the present context the phrasing "total content of the whole grain" is to be understood as the combination of the content of "hydrolyzed whole grain" and "solid whole grain content". If not indicated otherwise, "total content of the whole grain" is provided as % by weight in the final product. In an embodiment the hydrolyzed whole grain composition has a total content of the whole grain in the range of 1-99% by weight of the whole grain composition, such as 1-80%, such as 1-60%, such as 10-50%, such as 10-40% or such as 15-25%.

In the present context the phrasing "content of the hydrolyzed whole grain composition" is to be understood as the % by weight of hydrolyzed whole grains in the final product. Hydrolyzed whole grain composition content is part of the total content of the whole grain composition. Thus, in an embodiment the hydrolyzed whole grain composition according to the invention has a content of the hydrolyzed whole grain composition in the range 1-99% by weight of the whole grain composition, such as 1-80%, such as 1-60%, such as 10-50%, such as 10-40% or such as 15-25%.

The amount of the hydrolyzed whole grain composition in the final composition may depend on the type of whole grain component and the amount of added liquid during production. Similarly, if the product is dried the whole grain concentration will go up.

It would be advantageous to have a hydrolyzed whole grain composition comprising a high content of dietary fibers without compromising the organoleptic parameters of an end-product. Thus, in yet an embodiment the hydrolyzed whole grain composition has a content of dietary fibers in the range of 0.1-20% by weight of the hydrolyzed whole grain composition, such as in the range 0.1-20%, such as in the range 5-20%, preferably, in the range of 5-15%. A food product comprising a hydrolyzed whole grain composition according to the invention may be provided with high amounts of dietary fibers by the addition of the hydrolyzed whole grain composition provided by the present invention. This may be done due to the unique setup of the process according to the present invention.

Dietary fibers are the edible parts of plants that are not broken down by digestion enzymes. Dietary fibers are fermented in the human large intestine by the microflora. There are two types of fibers: soluble fibers and insoluble fibers. Both soluble and insoluble dietary fibers can promote a number of positive physiological effects, including a good transit through the intestinal tract which helps to prevent constipation, or a feeling of fullness. Health authorities recommend a consumption of between 20 and 35 g per day of fibers, depending on the weight, gender, age and energy intake.

Soluble fibers are dietary fibers that undergo complete or partial fermentation in the large intestine. Examples of soluble fibers from cereals include beta-glucans, arabinoxylans, arabinogalactans and resistant starch type 2 and 3, and oligosaccharides deriving from the latters. Soluble fibers from other sources include pectins, acacia gum, gums, alginate, agar, polydextrose, inulins and galacto-oligosaccharides for instance. Some soluble fibers are called prebiotics, because they are a source of energy for the beneficial bacteria (e.g. Bifidobacteria and Lactobacilli) present in the large intestine. Further benefits of soluble fibers include blood sugar control, which is important in diabetes prevention, control of cholesterol, or risk reduction of cardiovascular disease.

Insoluble fibers are the dietary fibers that are not fermented in the large intestine or only slowly digested by the intestinal microflora. Examples of insoluble fibers include celluloses, hemicelluloses, resistant starch type 1 and lignins. Further benefits of insoluble fibers include promotion of the bowel function through stimulation of the peristalsis, which causes the muscles of the colon to work more, become stronger and function better. There is also evidence that consumption of insoluble fibers may be linked to a reduced risk of gut cancer.

The moisture content of the hydrolyzed whole grain composition according to the invention may vary. Thus, in another embodiment the moisture content of the hydrolyzed whole grain composition is in the range of 1-50% by weight of the hydrolyzed whole grain composition, e.g. in the range of 20-40%. Examples of factors influencing the moisture content may be the amount of the hydrolyzed whole grain composition and the degree of hydrolysis in this composition. In the present context the phrasing "total solid content" equals 100 minus moisture content (%) of the composition.

The hydrolyzed whole grain composition according to the invention may be provided in different forms. Thus, in an embodiment the hydrolyzed whole grain composition is provided in the form of a liquid, a concentrate, a powder, a juice or a puree. An advantage of having hydrolyzed whole grain composition in different forms is that when used in a food product dilution may be avoided by using a dry or semi dry form. Similarly, if a more moisten product is desirable, a hydrolyzed whole grain composition in a liquid state may be used.

Humectants are often added to products which are to be in a dry or semi-dry state. Thus, in an embodiment the hydrolyzed whole grain composition does not comprise a humectant.

For the aspect of providing the hydrolyzed whole grain composition of the present invention a process is provided, said process comprising:

a) contacting a whole grain component with an enzyme composition in water, the enzyme composition comprising at least one alpha-amylase, said enzyme composition showing no hydrolytic activity towards dietary fibers,
b) allowing the enzyme composition to react with the whole grain component, to provide a whole grain hydrolysate,
c) providing the hydrolyzed whole grain composition by inactivating said enzymes when said hydrolysate has reached a viscosity comprised between 50 and 5000 mPa.s measured at 65°C.

In an embodiment the enzyme composition further comprises a protease or fragment thereof, which protease or fragment thereof shows no hydrolytic activity towards dietary fibers when in the active state. Similar, the enzyme composition may comprise an amyloglucosidase and/or and glucose isomerase according to the present invention.

Several parameters of the process may be controlled to provide the hydrolyzed whole grain composition according to the invention. Thus, in an embodiment step 1b) is performed at 30-100°C, preferably 50 to 85°C such as 30-70°C. In a further embodiment step 1b) is performed for 1 minute to 24 hours, such as 1 minute to 12 hours, such as 1 minute to 6 hours, such as 5-120 minutes. In yet an embodiment step 1b) is performed at 30-100°C for 5-120 minutes.
In yet a further embodiment step 1c) is allowed to proceed at 70-150°C for at least 1 second, such as 1-5 minutes, such as 5-120 minutes, such as 5-60 minutes. In an additional embodiment step 1c) is performed by heating to at least 90°C for 5-30 minutes.

In yet an embodiment the reaction in step 1c) is stopped when the hydrolysate has reached a viscosity comprised between 50 and 4000 mPa.s, such as between 50 and 3000 mPa.s, such as between 50 and 1000 mPa.s, such as between 50 and 500 mPa.s. In an additional embodiment viscosity is measured at TS 50.

In another embodiment the hydrolyzed whole grain composition in step 1) is provided when said hydrolysate has reached a viscosity comprised between 50 and 5000 mPa.s and a total solid content of 25-60%. By controlling viscosity and solid content the hydrolyzed whole grain may be provided in different forms.

In an additional embodiment the hydrolyzed whole grain composition in step 1c) is provided in the form of a liquid, a concentrate, a powder, a juice or a pure. An advantage of being able to provide the hydrolyzed whole grain composition in different forms is that it is possible to add hydrolyzed whole grain in high concentrations to a food product without the risk of diluting the product.

The above parameters can be adjusted to regulate the degree of starch degradation, the sugar profile, the total solid content and to regulate the overall organoleptic parameters of the final product.

To improve the enzymatic processing of the whole grain component it may be advantageous to process the grains before or after the enzymatic treatment. By grounding the grains a larger surface area is made accessible to the enzymes, and the process may be speeded up. In addition the organoleptic parameters may be improved by using a smaller particle size of the grains. In an additional embodiment the whole grains are roasted or toasted before or after enzymatic treatment. Roasting and toasting may improve the taste of the final product.

To prolong the storage time of the product several treatment can be performed. Thus, in an embodiment the process further comprises at least one of the following treatments: UHT, pasteurization, thermal treatment, retort and any other thermal or non-thermal treatments, such as pressure treatment. In a further embodiment the hydrolyzed whole grain composition is applied to an enclosure under aseptic conditions. In yet an embodiment the hydrolyzed whole grain composition product is applied to an enclosure under non-aseptic conditions, such as by retort or hot-for-hold.

A further aspect of the invention relates to a food product comprising a hydrolyzed whole grain composition according to the invention. The hydrolyzed whole grain composition may be present in such food product at a 1-99% by weight of the food product, such as 1-60%, such as 1-40% and such as 1-20%. The concentration may depend on the type of food product in which the composition is used.

It should be noted that embodiments and features described in the context of one of the aspects or embodiments of the present invention also apply to the other aspects of the invention.

All patent and non-patent references cited in the present application, are hereby incorporated by reference in their entirety.

The invention will now be described in further details in the following non-limiting examples.

EXAMPLES

Example 1 - Preparation of a hydrolyzed whole grain composition

Enzyme compositions comprising Validase HT 425L (alpha-amylase) optionally in combination with Alcalase 2.4 L (protease) were used for the hydrolysis of wheat, barley and oats.

Mixing may be performed in a double jacket cooker, though other industrial equipment may be used. A scraping mixer works continuously and scraps the inner surface of the mixer. It avoids product burning and helps maintaining a homogeneous temperature. Thus enzyme activity is better controlled. Steam may be injected in the double jacket to increase temperature while cold water is used to decrease it.

In an embodiment, the enzyme composition and water are mixed together at room temperature, between 10 and 25°C. At this low temperature, the enzymes of the enzyme composition have a very weak activity. The whole grain component is then added and the ingredients are mixed for a short period of time, usually less than 20 minutes, until the mixture is homogeneous.

The mixture is heated progressively or by thresholds to activate the enzymes and hydrolyse the whole grain component.

Hydrolysis results in a reduction of the viscosity of the mixture. When the whole grain hydrolysate has reached a viscosity comprised between 50 and 5000 mPa.s measured at 65°C and e.g. a total solid content of 25 to 60% by weight, the enzymes are inactivated by heating the hydrolysate at a temperature above 100°C, preferably by steam injection at 120°C.

Enzymes are dosed according to the quantity of total whole grain. Quantities of enzymes are different depending on the type of whole grain component, as protein rates are different. The ratio water/whole grain component can be adapted according to required moisture for the final liquid whole grain. Usually, the water/whole grain component ratio is 60/40. Percents are by weight.

Hydrolysed whole wheat	
Whole wheat flour	Substrate
Enzyme amylase	0.10% based on the substrate
Enzyme protease	0.05% based on the substrate

Hydrolysed whole barley	
Whole barley flour	Substrate
Enzyme amylase	0.10% based on the substrate
Enzyme protease	0.05% based on the substrate

Hydrolysed whole oats	
Whole oats flour	Substrate
Enzyme amylase	0.10% based on the substrate
Enzyme protease	0.05% based on the substrate

Example 2 - Sugar profile of the hydrolyzed whole grain composition

Hydrolyzed whole grain compositions comprising wheat, barley and oat were prepared according to the method in example 1.

Carbohydrates HPAE:

The hydrolyzed whole grain compositions were analysed by HPAE for illustrating the sugar profile hydrolysed whole grain composition.

Carbohydrates are extracted with water, and separated by ion chromatography on an anion exchange column. The eluted compounds are detected electrochemically by means of a pulsed amperometric detector and quantified by comparison with the peak areas of external standards.

Total dietary fibres:

Duplicate samples (defatted if necessary) are digested for 16 hours in a manner that simulates the human digestive system with 3 enzymes (pancreatic alpha-amylase, protease, and amyloglucosidase) to remove starch and protein. Ethanol is added to precipitate high molecular weight soluble dietary fibre. The resulting mixture is filtered and the residue is dried and weighed. Protein is determined on the residue of one of the duplicates; ash on the other. The filtrate is captured, concentrated, and analyzed via HPLC to determine the value of low molecular weight soluble dietary fibre (LMWSF).

Whole wheat:

	Wheat Reference	**Wheat Hydrolysed Alcalase/Validase**
Total sugars (% w/w))	2.03	24.36
Glucose	0.1	1.43
Fructose	0.1	0.1
Lactose (monohydrate)	<0.1	<0.1
Sucrose	0.91	0.69
Maltose (monohydrate)	0.91	22.12
Mannitol	<0.02	<0.02
Fucose	<0.02	<0.02
Arabinose	<0.02	0.02
Galactose	<0.02	<0.02
Xylose	<0.02	<0.02
Mannose	<0.02	<0.02
Ribose	<0.02	<0.02
Insoluble and soluble fibers	12.90	12.94
LMW fibers	2.63	2.96
Total fibers	15.53	15.90

Whole oats:

	Oats Reference	**Oats Hydrolysed Alcalase/Validase**
Total sugars (% w/w))	1.40	5.53
Glucose	0.1	0.58
Fructose	0.1	0.1
Lactose (monohydrate)	<0.1	<0.1
Sucrose	1.09	1.03
Maltose (monohydrate)	0.11	3.83
Mannitol	<0.02	<0.02
Fucose	<0.02	<0.02
Arabinose	<0.02	<0.02
Galactose	<0.02	<0.02
Xylose	<0.02	<0.02
Mannose	<0.02	<0.02
Ribose	<0.02	<0.02
Insoluble and soluble fibers	9.25	11.28
LMW fibers	0.67	1.21
Total fibers	9.92	12.49

Whole Barley:

	Barley Reference	**Barley Hydrolysed Alcalase/Validase**
Total sugars (% w/w))	1.21	5.24
Glucose	0.1	0.61
Fructose	0.1	0.1
Lactose (monohydrate)	<0.1	<0.1
Sucrose	0.90	0.88
Maltose (monohydrate)	0.11	3.65
Mannitol	<0.02	<0.02
Fucose	<0.02	<0.02
Arabinose	<0.02	<0.02
Galactose	<0.02	<0.02
Xylose	<0.02	<0.02
Mannose	<0.02	<0.02
Ribose	<0.02	<0.02
Glucose	0.1	0.61
Fructose	0.1	0.1
Insoluble and soluble fibers	9.70	10.44
LMW fibers	2.23	2.63
Total fibers	11.93	13.07

The results clearly demonstrate that a significant increase in the glucose content is provided by the hydrolysis where the glucose content of the hydrolysed barley is 0.61% (w/w) on a dry matter basis; the glucose content of the hydrolysed oat is 0.58% (w/w) on a dry matter basis; and the glucose content of the hydrolysed wheat is 1.43% (w/w) on a dry matter basis.

Furthermore, the results also demonstrates that the maltose:glucose ratio is ranging from about 15:1 to about 6:1.

Thus, based on these results a new sugar profile is provided having an increased sweetness compared to the prior art.
In conclusion, an increased sweetness may be obtained by using the hydrolyzed whole grain composition according to the invention and therefore the need for further sweetening sources may be dispensed or limited.

In addition, the results demonstrate that the dietary fiber content is kept intact and the ratio and amount of soluble and insoluble fibers are substantially the same in the non-hydrolyzed whole grain and in the hydrolyzed whole grain composition.

Example 3 – Hydrolytic activity on dietary fibers

The enzymes Validase HT 425L (Valley Research), Alcalase 2.4L (Novozymes) and BAN (Novozymes) were analysed using a thin layer chromatography analysis for activity towards arabinoxylan and beta-glucan fibre extracts both components of dietary fibers of whole grain.

The results from the thin layer chromatography analysis showed that the amylase Validase HT and the protease Alcalase showed no hydrolytic activity on either beta-glucan or arabinoxylan, while the commercial alpha-amylase preparation, BAN, causes hydrolysis of both the beta-glucan and arabinoxylan, see figure 1. See also example 4.

Example 4 - Oat β-Glucan and Arabinoxylan molecular weight profile following enzymatic hydrolysis

Hydrolysis:

A solution of 0.5 % (w/v) of Oat β-Glucan medium viscosity (Megazyme) or Wheat Arabinoxylan medium viscosity (Megazyme) was prepared in water.
The enzyme was added at an enzyme to substrate ratio (E/S) of 0.1 % (v/v). The reaction was allowed to proceed at 50°C for 20 minutes, the sample was then placed at 85°C during 15 min to enable starch gelatinization and hydrolysis. The enzymes were finally inactivated at 95°C for 15 minutes. Different batches of the following enzymes have been evaluated.

Alcalase 2.4L (Valley Research):	batch BN 00013
	batch 62477
	batch 75039
Validase HT 425L (Valley Research):	batch RA8303A
	batch 72044
MATS L (DSM):	batch 408280001

Molecular weight analysis
Hydrolyzed samples were filtered on a syringe filter (0.22 µm) and 25 µL were injected on a High Pressure Liquid Chromatography Agilent 1200 series equipped with 2 TSKgel columns in serie (G3000PWXL 7,8 x 300 mm), (GMPWXL 7,8X 30 mm) and with a guard column (PWXL 6 x 44 mm). (Tosoh Bioscence)
Sodium Nitrate 0.1M/ at 0.5ml/min was used as running buffer. Detection was done by reflective index measurement.

Results
On figures 2-4 graphs for both a control (no enzyme) and test with enzymes are plotted. However, since there are substantially no difference between the graphs it may be difficult to differentiate both graphs from each other.

Conclusions
No shift in oat beta glucan and wheat arabinoxylan fibre molecular weight profile was determined following hydrolysis with the Alcalase 2.4 L (figure 2), Validase HT 425 L (figure 3) or MATS L (figure 4).

Claims

1. A hydrolyzed whole grain composition comprising an alpha-amylase or fragments thereof, which alpha-amylase or fragments thereof show no hydrolytic activity towards dietary fibers when in the active state, and wherein the hydrolyzed whole grain composition has:

a content of glucose of at least 0.25% by weight of the hydrolysed whole grain composition on a dry matter basis, and/or
a maltose to glucose ratio below 144:1 by weight in the composition, or
a maltose to fructose ratio below 230:1 by weight in the composition, or
a maltose to glucose+fructose ratio below 144:1 by weight in the composition.

2. The hydrolyzed whole grain composition according to claim 1, further comprising a protease or fragment thereof, at a concentration of 0.001-5% by weight of the total whole grain content, which protease or fragment thereof shows no hydrolytic activity towards dietary fibers when in the active state.

3. The hydrolyzed whole grain composition according to claim 2, wherein 1-10% of the proteins from the whole grain composition is hydrolyzed, such as 2-8%, e.g. 3-6%, 10-99%, such as 30-99%, such as 40-99%, such as 50-99%, such as 60-99%, such as 70-99%, such as 80-99%, such as 90-99%, or such as 10-40%, 40-70%, and 60-99%.

4. The hydrolyzed whole grain composition according to any of the preceding claims, with the proviso that it does not comprise a beta-amylase.

5. The hydrolyzed whole grain composition according to any of claims 1 and 4, with the proviso that it does not comprise the protease.

6. The hydrolyzed whole grain composition according to any one of the preceding claims, wherein the composition further comprises an amyloglucosidase or fragments thereof, which amyloglucosidase or fragments thereof show no hydrolytic activity towards dietary fibers when in the active state.

7. The hydrolyzed whole grain composition according to any one of the preceding claims, wherein the composition further comprises a glucose isomerase or fragments thereof, which glucose isomerase or fragments thereof show no hydrolytic activity towards dietary fibers when in the active state.

8. The hydrolyzed whole grain composition according to any of the preceding claims, wherein the hydrolyzed whole grain composition has a substantial intact beta-glucan structure relative to the starting material.

9. The hydrolyzed whole grain composition according to any of the preceding claims, wherein the hydrolyzed whole grain composition has a substantial intact arabinoxylan structure relative to the starting material.

10. The hydrolyzed whole grain composition according to any of the preceding claims, having a content of dietary fibers in the range 0.1-20% by weight of the hydrolyzed whole grain composition.

11. The hydrolyzed whole grain composition according to any one of the preceding claims, wherein the hydrolyzed whole grain composition is provided in the form of a liquid, a concentrate, a powder, a juice or a puree.

12. A process for preparing a hydrolyzed whole grain composition according to any of claims 1-11, said process comprising:

a) contacting a whole grain component with an enzyme composition in water, the enzyme composition comprising at least one alpha-amylase, said enzyme composition showing no hydrolytic activity towards dietary fibers,
b) allowing the enzyme composition to react with the whole grain component, to provide a whole grain hydrolysate,
c) providing the hydrolyzed whole grain composition by inactivating said enzymes when said hydrolysate has reached a viscosity comprised between 50 and 5000 mPa.s measured at 65°C.

13. The process according to claim 12, wherein the enzyme composition further comprises a protease or fragments thereof, which protease or fragments thereof shows no hydrolytic activity towards dietary fibers when in the active state;

14. The process according to any of claims 12 or 13, wherein the step 1c) is performed by heating to at least 90°C for 5-30 minutes.

15. A food product comprising a hydrolyzed whole grain composition according to any of claims 1-11.

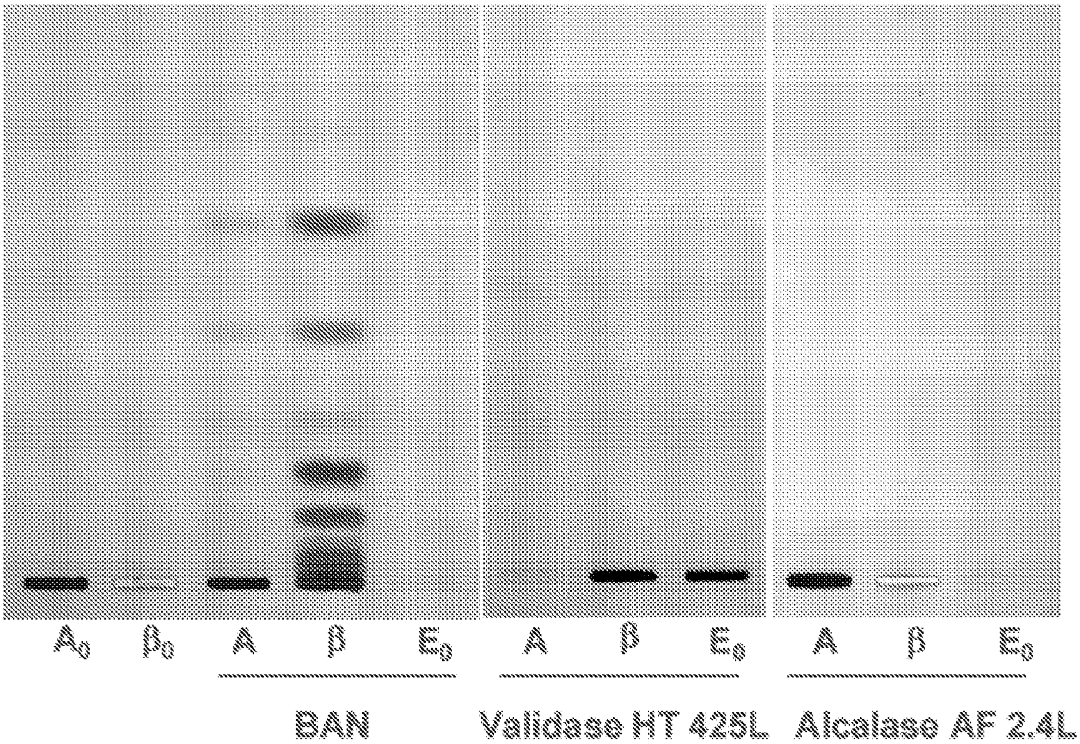

Fig. 1

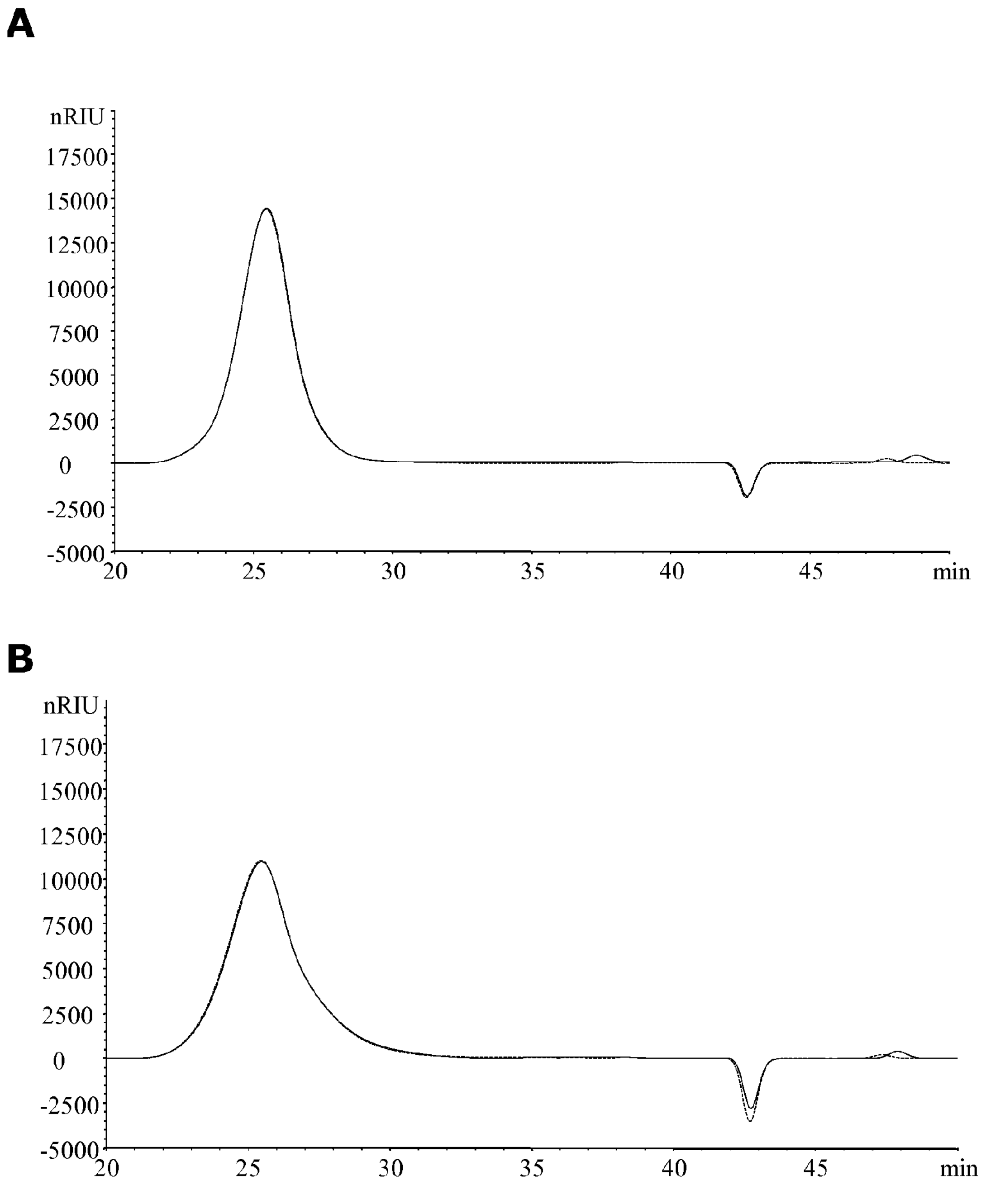
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Fig. 2

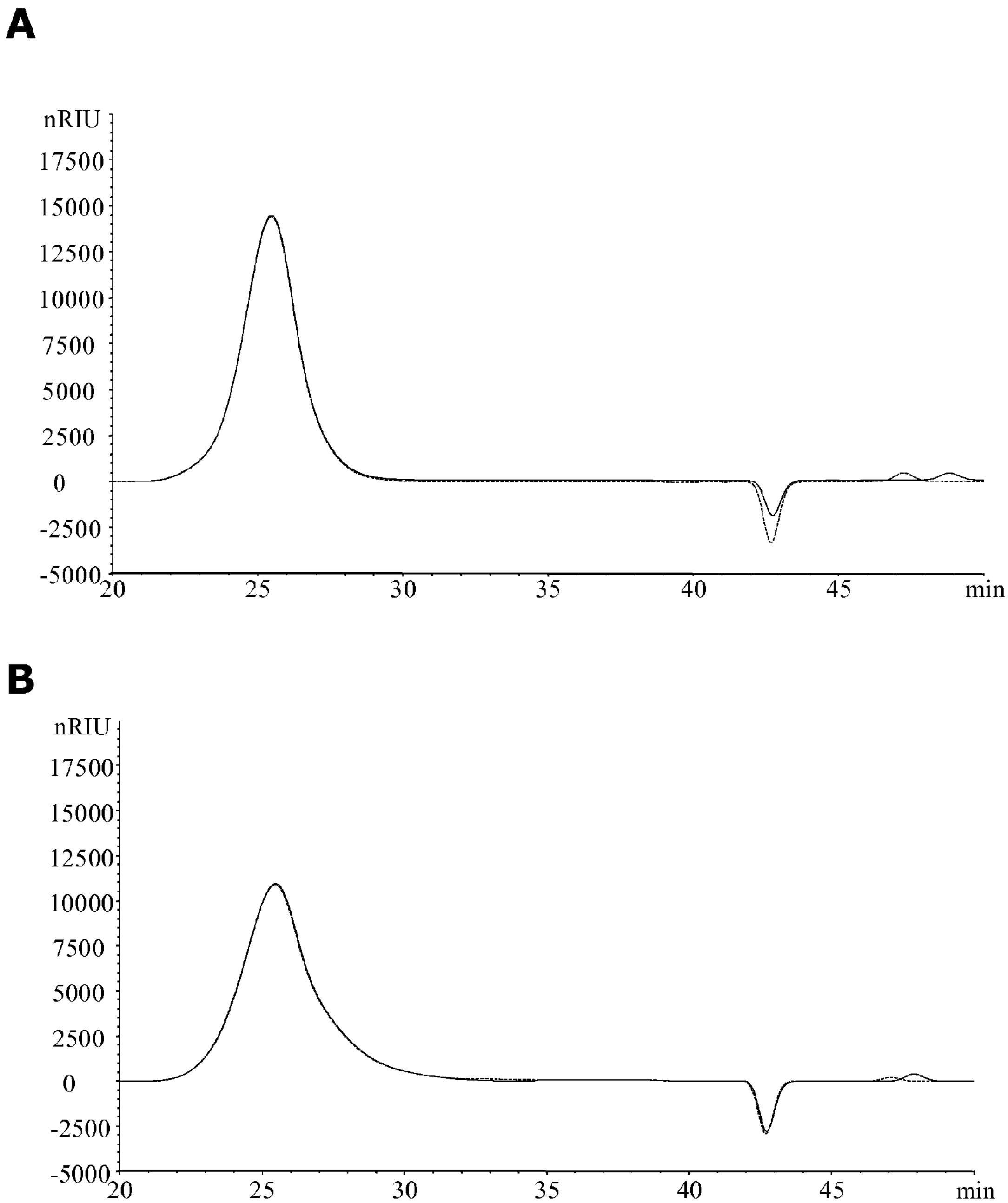
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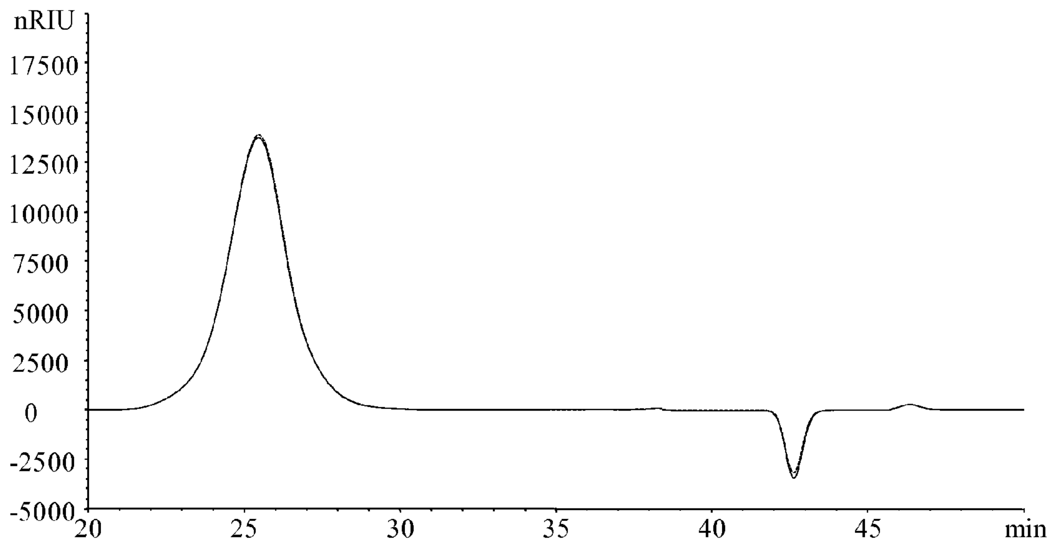
Fig. 3

A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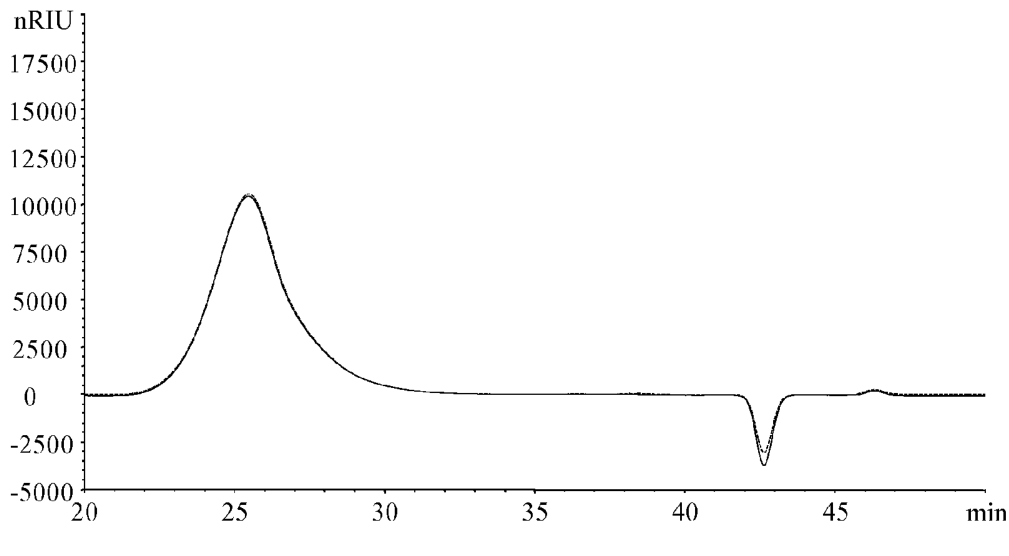
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Fig.4