

GLUTEN ENHANCER**FIELD OF THE INVENTION**

This invention relates to enhancing gluten formation in flours, doughs and baked products, and more specifically to baking enzyme compositions to enhance gluten formation in doughs and baked products to improve the baking performance of flour.

BACKGROUND OF THE INVENTION

Baking flour is milled from cereal grains such as wheat, barley, buckwheat, oat, maize, rice, oat, triticale, rye, and mixtures thereof. Wheat flour has the highest protein content of all the cereal grains and therefore typically forms the basis for leavened baked products. The gluten protein composite of gliadin and glutenin is found in the endosperm of cereal grain kernels and is the key factor in the baking process. Upon hydration, gliadin and glutenin bonds together and form a matrix or network of gluten strands to form the structure that encompasses other components and provides the gas retaining capabilities of leavened baked products. In order for the gluten protein composite to fulfil this role, the protein must be vital, in that the gluten protein composite must not be denatured to form gluten when mixed with water.

The protein quality and content present in the cereal grain kernel is directly reliant on the weather and growing conditions in which the cereal grain crops are grown. For example, in extremely wet weather conditions during the harvest season, protein levels in cereal grains are considerably reduced. For example, protein content of available wheats may typically be reduced by 2 to 2.5% either due to unavailability of high protein wheats due to weather damage/destruction and/or reduced soil nitrogen (due to leaching). This typically results in the final flour overall protein content reduction of 1.0 to 1.5%. The functional gluten-forming proteins are as a consequence also reduced. Accordingly, the baking performance of the milled bakers flour product produced from these cereal grains harvested during extremely wet weather is compromised.

In an attempt to improve or enhance the baking performance of flour, there is a continuous effort to develop flour agents and dough conditioners. These baking aids are commercially available and commonly added to improve specific properties of the baking process, for example to adjust gluten strength, loaf volume, elasticity, machinability, crust crispiness, crumb structure, flavor, yeast fermentation, water absorption-pH-hardness, staling and shelf life. Such dough conditioners include emulsifiers such as calcium stearyl-2-lactylate (CSL), sodium stearyl lactylate (SSL), diacetyl tartaric acid ester of mono- and diglycerides (DATEM), or the like. Salts and acids such as calcium carbonate, monocalcium phosphate, for example, alter the pH and water hardness in the mixture.

Other dough conditioners include ammonium salts and enzymes such as amylase to improve yeast formation, and maturing or oxidizing agents such as potassium bromate, ascorbic acid, azodicarbonamide (ADA), potassium iodate for increasing gluten strength. Another conventional dough conditioner is vital wheat gluten that is typically added to flour to improve flour quality and increase the protein content.

Adding baking aids in the baking process increases the overall baking costs. Additionally, there is a growing consumer driven demand to replace chemical additives with more natural products, such as enzyme compositions. For example, potassium bromate is now banned as a flour treatment agent or baking additive in most countries. Potassium iodate and azodicarbonamide (ADA) are also not permitted as flour treatment agents or baking additives in some countries including Singapore.

In the situation where the quality and content of the endogenous or native protein of an entire harvest is compromised by extremely wet weather conditions, the conventional method of the remedying the situation in the milling and baking industries is to add supplemental vital wheat gluten to the milled flour to supplement the inherently lower and weaker protein content of the endogenous protein to re-establish acceptable baking performance of the flour. The supplemental protein of the added vital wheat gluten has the ability to enter into and increase the vitality of the existing endogenous gluten-forming protein framework. However, as the refining process for producing vital wheat gluten is costly, the addition of vital wheat gluten as a baking aid poses a substantial cost impact in the milling and baking industry.

There is a need to provide a less costly alternative to conventional baking aids such as vital wheat gluten that naturally enhance the gluten formation in flours, doughs and baked products.

SUMMARY OF THE INVENTION

An aspect of the invention is a baking enzyme composition comprising a lipase, an oxido-reductase, a xylanase and a transferase.

In an embodiment, the baking enzyme composition is derived from microbial origin.

In an embodiment, the lipase is derived from *Aspergillus niger*, *Bacillus subtilis*, *Aspergillus oryzae*, *Rhizopus oryzae*, or *Fusarium oxysporum*.

In an embodiment, the oxido-reductase is glucose oxidase.

In an embodiment, the oxido-reductase is derived from *Aspergillus niger*.

In an embodiment, the xylanase is fungal xylanase or beta-glucanase.

In an embodiment, the xylanase is derived from *Aspergillus oryzae* or *Aspergillus niger*.

In an embodiment, the transferase is transglutaminase.

In an embodiment, the transferase is derived from *Aspergillus niger* or *Streptomyces mobaraensis*.

In an embodiment, the lipase is between about 5 to 200 units per kilogram of flour; the oxido-reductase is between about 5 to 1000 units per kilogram of flour; the xylanase is between about 10 to 1000 units per kilogram of flour; and the transferase is between about 0.1 to 5 units per kilogram of flour.

In an embodiment, the lipase is between about 15 to 90 units per kilogram of flour; the oxido-reductase is between about 70 to 420 units per kilogram of flour; the xylanase is between about 55 to 330 units per kilogram of flour; and the transferase is between about 0.1 to 1.2 units per kilogram of flour.

An aspect of the invention is a pre-mix comprising a flour having an endogenous gluten forming protein; and the baking enzyme composition.

An aspect of the invention is a dough comprising a baking enzyme composition.

An aspect of the invention is a method to prepare a dough comprising adding to dough comprising flour, water, yeast, and the baking enzyme composition or pre-mix.

An aspect of the invention is a method of enhancing the endogenous vital gluten content of a flour comprising adding the baking enzyme composition.

An aspect of the invention is a food product comprising the baking enzyme composition.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings incorporated herein and forming a part of the specification illustrate several aspects of the present invention and, together with the description, serve to explain the principles of the invention. While the invention will be described in connection with certain embodiments, there is no intent to limit the invention to those embodiments described. On the contrary, the intent is to cover all alternatives, modifications and equivalents as included within the scope of the invention as defined by the appended claims. In the drawings:

FIG. 1 is an illustration showing the combined effect of glucose oxidase and xylanase in accordance with an embodiment of the invention;

FIG. 2 is an illustration showing the combined effect of glucose oxidase and xylanase of FIG. 1 in more detail in accordance with an embodiment of the invention;

FIG. 3 is a graph showing a farinogram reading relating to EXAMPLE I of mixing of dough prepared from Sample 1 - control flour;

FIG. 4 is a graph showing a farinogram reading relating to EXAMPLE I of mixing of dough prepared from Sample 2 - control flour plus 1% vital wheat gluten;

FIG. 5 is a graph showing a farinogram reading relating to EXAMPLE I of mixing of a dough prepared from Sample 3 - control flour plus 500 ppm baking enzyme composition in accordance with an embodiment of the invention;

FIG. 6 is a graph showing an extensogram reading relating to EXAMPLE I of mixing of dough prepared from Sample 1 - control flour;

FIG. 7 is a graph showing an extensogram reading relating to EXAMPLE I of mixing of a dough prepared from Sample 2 - control flour plus 1 % vital wheat gluten;

FIG. 8 is a graph showing an extensogram reading relating to EXAMPLE I of mixing of a dough prepared from Sample 3 - control flour plus 500ppm baking enzyme composition in accordance with an embodiment of the invention;

FIG. 9 shows the external baked product results relating to EXAMPLE II including a baked product with the baking enzyme composition in accordance with an embodiment of the invention;

FIG. 10 shows the internal baked product results relating to EXAMPLE II including a baked product with the baking enzyme composition in accordance with an embodiment of the invention;

FIG. 11 is a graph 180 showing the results of EXAMPLE II of the specific volume versus addition of vital wheat gluten curve 182 and the baking enzyme composition curve 184 in accordance with an embodiment of the invention; and

FIG. 12 shows the external baked product results of doughs shocked by drop test after final proof relating to EXAMPLE III including a baked product with the baking enzyme composition in accordance with an embodiment of the invention.

DETAILED DESCRIPTION

A baking enzyme composition, and method for making the same, is disclosed to enhance the functional properties of the intrinsic gluten-forming proteins of baking flour in doughs and baked

products to improve the baking performance of baking flour. The baking enzyme composition is a combination of enzymes having an action (or dough improving effect) like that of additional vital wheat gluten. The baking enzyme composition in accordance with an embodiment of the invention relates to methods for preparing a flour, a dough, a baked, fried, steamed, or the like product resulting from the flour or dough, comprising an effective amount of baking enzyme composition in accordance with an embodiment of the invention which improves one or more properties of the flour, dough, product resulting from the flour or dough, or the like, relative to a flour, a dough, or a baked product which the baking enzyme composition in accordance with an embodiment of the invention is not incorporated.

In an embodiment, the functionality of the baking enzyme composition strengthens and improves the baking performance of baking flour having traces of gluten protein composite of gliadin and glutenin. The baking enzyme composition strengthens and improves the baking performance of wheat flour and is used as a vital wheat gluten replacer introduced into the flour at different stages of the baking process including at the flour mill, the bakery, or the like. The baking enzyme composition strengthens baking flour that has been compromised by unfavourable crop growing conditions, such as in extremely wet weather conditions during the harvest season. The baking enzyme composition supplements and enhances the action of existing (endogenous) gluten-forming proteins (glutenin and gliadin).

The baking enzyme composition in accordance to an embodiment of the invention replaces vital wheat gluten as a baking aid. In addition to being a gluten strengthening agent of flour in the baking of breads, buns, and the like, the baking enzyme composition is also a gluten strengthening agent to replace added vital wheat gluten in noodle production. The baking enzyme composition increases the elasticity of the cooked noodle product by a minimum of 10 per cent compared to the elasticity of noodle product containing the added vital wheat gluten.

After experimentation, it has been found that a significant strengthening effect of the gluten network in wheat flour dough can be achieved by use of a complex enzyme mixture of the baking enzyme composition in accordance with an embodiment of the invention consisting of a hemicellulase and at least one enzyme selected from the group consisting of glucose oxidase, lipase and transglutaminase. It has also been found that up to 4% added vital wheat gluten can be replaced in preparation of dough for bread-making by using this complex mixture. Furthermore, it has been found in most cases, that dough water absorption is able to be substantially maintained. The experiments conducted were designed to compare the improving effects of addition of various levels of vital wheat gluten to bread dough based on a general purpose flour with 10.5% protein

content versus various dosages of the enzyme combination of enzymes. The results confirm that up to 4% of added vital wheat gluten can be replaced by the combination of the baking enzyme composition without any significant change in dough processing, dough water absorption or loaf volume. The result was surprising, in regard to both the extent of improvement of product particularly in terms of maintenance of water absorption and loaf volume relative to that obtained with addition of vital wheat gluten.

Certain combinations of enzymes can result in certain synergistic effects especially when used in a complex environment such as the gluten matrix within the microstructure of bread dough. The baking enzyme composition in accordance with an embodiment of the invention has been developed to take advantage of such synergies by combining enzyme activities that have been found to strengthen the gluten structure. For example, glucose oxidase combined with hemicellulase can result in oxidative gelation of arabinoxylans in the dough thus improving dough visco-elastic properties that may lead to improvement of oven spring and loaf volume, which is discussed in further detail below with reference to FIG. 1 and FIG. 2. Furthermore, some of these enzymes are prepared by a fermentation process that results in a range of side activities, which is believed to further enhance the baking performance. Several of the enzymes used in the baking enzyme composition are prepared using conventional submerged fermentation or solid state fermentation (SSF) production methods with non-genetically modified organisms (non-GMO) production organisms which by their very nature result in minor side activities being produced in addition to the principle activity, for example, cellulase, beta-glucanase, protease, and the like. The baking enzyme composition has advantages over existing baking aids or dough improvers. The baking enzyme composition has the ability to fully replace at least up to 3% exogenous / added vital wheat gluten without compromising dough water absorption or dough machinability.

The baking enzyme composition in accordance with an embodiment of the invention comprises a complex enzyme mixture of microbial origin selected from the groups of enzyme activities known as lipases, oxido-reductases, xylanases and transferases.

Lipases:

Suitable sources of lipases in accordance with an embodiment of the invention may be obtained from *Aspergillus niger*, *Bacillus subtilis*, *Aspergillus oryzae*, *Rhizopus oryzae*, *Fusarium oxysporum*. The lipase complex in the baking enzyme composition in accordance with an embodiment of the invention is able to hydrolyse the neutral triglycerides into monoglycerides and the galacto and phospholids into galactomonoglycerides and lyso-phospholipids, which have a dough strengthening

effect similar to that of dough-conditioning emulsifiers, such as calcium stearoyl-2-lactylate (CSL), sodium stearoyl lactylate (SSL), diacetyl tartaric acid ester of mono- and diglycerides (DATEM). Side activities associated with at least one of the sources of lipase used include beta-glucanase, which has a possible impact on dough rheology in line with the presence of variable levels of beta-glucans in wheat flour and acid alpha amylase, which is synergistically active with fungal-alpha amylase to improve supply of maltose to yeast.

Oxido-reductases:

Suitable oxido-reductases in accordance with an embodiment of the invention are from the subgroup glucose oxidase, for which the preferred source is *Aspergillus niger*. Glucose oxidase is an enzyme that uses oxygen to oxidise glucose to gluconic acid and hydrogen peroxide. In a bread dough, the hydrogen peroxide that is formed is able to then oxidize and couple two cysteine residues or –SH groups that are adjacent within a gluten matrix. The resultant disulphide or –S-S- bonds that are formed cross-link the polypeptides or gluten chains within the bread dough. This cross-linking by formation of disulphide bond is similar to that achieved by chemical oxidants such as dehydroascorbic acid or azodicarbonamide. Another reaction that may occur in bread dough as a result of use of glucose oxidase is an oxidative gelation reaction thought to occur through cross-linking of water soluble arabinoxylans by oxidation of their ferulic acid residues. Combination with hemicellulase or xylanase results in a synergy that stabilizes the dough water distribution and rheological properties within the microstructure of the gluten matrix of the dough.

Xylanases:

Suitable xylanases in accordance with an embodiment of the invention include fungal xylanases, such as xylanase from *Aspergillus oryzae* and *Aspergillus niger*. Xylanases solubilize insoluble pentosans within the hemicelluloses fraction of the non-starch polysaccharides of wheat flour, primarily through cleavage of the arabinoxylan polymer at the endo-1,4-beta site. This produces a much more stable dough with improved gas retention due to the reduction in the proportion of insoluble xylans that can interfere with gas retention properties and stability of the gluten structure within the dough. Side activities associated with the source of xylanase in the baking enzyme composition in accordance with an embodiment of the invention include beta-glucanase which has a further possible impact on the rheology of the dough. Beta-glucanase provides improved crumb structure and stability, particularly in rye breads, or breads containing oat or barley flours that contain relatively high levels of beta glucans. Other minor activities such as protease, amylase other

arabinoxylanosidases may improve dough extensibility, dough tolerance, and gas production and retention.

Transferases:

Suitable transferases in accordance with an embodiment of the invention include the enzyme commonly referred to as transglutaminase. Suitable microbial sources are *Aspergillus niger* and *Streptomyces mobaraensis*. A transglutaminase is an enzyme that catalyzes the intermolecular and/or intramolecular cross-linking of proteins. Specifically, transglutaminases catalyze the formation of a covalent bond between a free amine group of a lysine and a gamma-carboxamide group of glutamine. In the case of wheat gluten, the use of transglutaminase forms a covalent bond that cross-links chains of gluten proteins, thus resulting in a dough strengthening effect.

In accordance with an embodiment of the invention, a baking enzyme composition with the desired improving effect provides between about 10 and 1000 Units of xylan degrading enzyme, about 5 to 200 units of lipid degrading enzyme per Kg of flour, about 5 to 1000 units of glucose oxidase per Kg of flour and about 0 to 5 units of transglutaminase per Kg of flour. The baking enzyme composition in accordance with an embodiment of the invention may have a dosage such as:

Glucose Oxidase:	70 to 420 GOX U/Kg of flour;
Lipid degrading enzymes:	15 to 90 PHOS/Kg of flour;
Xylan degrading enzymes:	55 to 330 AXU/Kg of flour; and
Transglutaminase:	0.2 to 1.2 TGU/Kg of flour.

One glucose oxidase (GOX) unit is defined to correspond to the amount of enzyme which under the specified conditions results in the conversion of 1 μ mole glucose per min. The activity is stated as units per g of enzyme preparation.

One lipid degrading enzyme unit (PHOS) is defined to correspond to the amount of enzyme needed to liberate 1 μ mol free fatty acid per minute from phosphatidylcholine at pH 4 and 37°C .

One acid xylanase unit (AXU) is defined as the quantity of enzyme that when diluted in one ml and used in assay conditions at 30 degrees Celsius and pH 2.75, will liberate oligomers from Xylan (Oat) spelt dyed with Remazol brilliant blue so that the absorbance of supernatant measured at 520 nm is 0.2. Remazol brilliant blue is available commercially from Megazyme International Ireland, Wicklow Ireland where REMAZOL is a trademark of DyStar Colours Distribution GmbH, Frankfurt, Germany

The transglutaminase activity of an enzyme preparation can be determined by means of the colorimetric hydroxamate test. Here one transglutaminase unit (TGU)/g is defined as the quantity of an enzyme which

under standardized conditions, at 37 degree Celsius and pH 6.0 with 0.2 M tris-HCl buffer, releases 1 μ mol hydroxyamine acid.

It will be appreciated that the units of enzyme activity referred to in terms of “per Kg of flour” are the range of dosages of the various enzymes (in terms of their respective units of activities) that may be provided by the baking enzyme composition for each Kg of final flour, either as, for example, produced by the miller, used by the baker, or the like. It will be appreciated that the activity units have values that represent the relative dosage in terms of the specific units of activity of the different enzymes in the baking enzyme composition. As the different activity units are based on the particular methods of analysis used for each specific type of enzyme, these values are not directly proportional to the masses of the different types of enzymes or enzyme proteins in the enzyme baking composition.

It will be understood that flour is not an essential element within the baking enzyme composition per se. In some embodiments, flour may be used as part of a neutral carrier/bulking agent for practicability. The premixed baking enzyme composition may also comprise, in addition to the enzymes, a neutral carrier such as wheat flour or the like to enable practical dosing and to ensure proper dispersion.

It will be appreciated that some of these enzymes have, either in isolation or in certain combinations, been proposed as bread improver agents, but it has been found in experiments that the combined action of the constituent enzymes in the baking enzyme composition in accordance with an embodiment of the invention has a cumulative improving effect that is superior to that if any one of the component enzymes is deleted. Enzymes such as certain xylanases and phospholipase contribute to improved dough fermentation- tolerance, loaf volume and improved crumb structure when used either alone or in combination with other enzymes or bread improver components such as alpha- amylase, l-ascorbic acid and certain emulsifiers such as DATEM (Diacetyl Tartaric Acid Esters of Mono and diglycerides or SSL (Sodium Stearoyl-2-lactylate). Because the gluten matrix in a bread dough is a very complex structure, the baking performance of which is largely but not solely dependent on the quantity and innate quality of gluten-forming proteins. Baking performance may also be influenced by the structure and form of other flour components such as hemicellulose, lipids, as well as the development and maturity of the dough through fermentation, physical dough development (mixing) and presence of maturing agents in the dough such as oxygen, ascorbic acid, oxido-reductase enzymes such as glucose oxidase.

The combination of enzymes of the baking enzyme composition in accordance with an embodiment of the invention is composed of various types of enzymes that function both by strengthening the endogenous gluten-forming proteins by at least two mechanisms (sulphydryl bridge formation and cross-linking via lysine and glutamine groups) and by suitably modifying lipids and hemicellulose of

the flour to further enhance the microstructure of the gluten matrix in the dough. If any one of the components is deleted from the baking enzyme composition in accordance with an embodiment of the invention, the particular mechanism that would otherwise be available to help support enhanced gluten microstructure would be lacking, thereby compromising baking performance.

FIG. 1 and FIG. 2 are illustrations 10,20 showing the combined effect of glucose oxidase and xylanase in accordance with an embodiment of the invention. FIG. 1 and FIG. 2 illustrate a hypothesis model for the combined (synergistic) effect of glucose oxidase 12 and xylanase 14 as discussed in Primo-Martin et al., *J Sci Food Agric* 85:1186–1196 (2005), the content of which is incorporated herein by reference. FIG. 1 generally shows that glucose oxidase 12 effects disulphide bridge formation 16 via oxidation of sulphhydryl groups 17 of gluten 18, and FIG. 2 shows in more detail that glucose oxidase 12 effects disulphide bridge formation 14 via oxidation of sulphhydryl groups 17 of gluten 18 and also oxidation of arabinoxylans (AX) 22.

It will be appreciated that if glucose oxidase 12 is used alone, i.e. without xylanase (route 1 in FIG. 2), oxidative cross-linking of large or high molecular weight arabinoxylans 22 or native arabinoxylans occurs via ferulic acid 24 residues to form very high molecular weight large polymers of arabinoxylans (AX) 26. Route 1 is followed in the absence of xylanase, high molecular weight AX 22 is crosslinked with the formation of large polymers of arabinoxylans 26. The large polymers of arabinoxylans 26 that are formed may interfere with the structure of the gluten matrix in bread doughs. This could have potentially negative effects on product quality. This is despite the otherwise positive effect of glucose oxidase in its primary role of strengthening gluten 18 via formation of disulphide bridging 14 of gluten 18.

Route 2 in FIG. 2 is followed in the presence of xylanase 28, leading to small AX fragments 30, or larger AX fragments 32, and either crosslinked small AX fragments 34 or small AX fragments cross-linked to high molecular weight AX 36 that form marginally larger AX polymers.

There is a correction effect by xylanase 14 due to the cleaving of AX backbone and leading to either cross-linked small AX fragments 34 or small AX fragments cross-linked to native AX molecules 36 which lead to only marginally larger polymers. This correction effect overcomes the potential interfering effects of very high molecular weight arabinoxylans 26 on the gluten matrix.

It will be appreciated that sufficient functional “vital” gluten-forming proteins are present in the flour or dough for the baking enzyme composition in accordance with embodiments of the invention to function and enhance baking performance. If the flour does not contain gluten naturally, then gluten may be added at a level required to achieve the dough properties and final product

characteristics required. If the baking enzyme composition in accordance with an embodiment of the invention is added with gluten, then less gluten will be required to achieve the desired dough properties and final desired product characteristics by using the baking enzyme composition.

Sufficient total amount of gluten-forming proteins is required either in the flour or added to the flour in order produce bread of acceptable quality. The baking enzyme composition in accordance with an embodiment of the invention functions as a gluten enhancer, and a sufficient level of endogenous gluten-forming protein is present. In bread baking, it is not only quantity of protein that impacts bread quality but also the "quality" or characteristics of the gluten-forming proteins. If there is insufficient gluten-forming protein of good baking quality in the flour, the baker or miller may choose to add vital wheat gluten to supplement the endogenous protein in order to achieve desired product quality. The baking enzyme composition can enhance the gluten structure in bread dough to provide the baker or miller an alternative to supplementation with vital wheat gluten.

It will be appreciated that there are various analytical methods commonly used to measure crude protein content of cereal flours, such as for example, near-infrared light (NIR) spectroscopy, Kjeldahl nitrogen determination, combustion analyses based on the Dumas or modified Dumas method, performed on instrumentation commercially available from Leco Corporation, Michigan, United States of America, or the like. Gluten-forming proteins may be quantitatively measured by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for the analysis of the quantitative composition of proteins.

The gluten enhancer of the baking enzyme composition in accordance with an embodiment of the invention may be either added to the flour or added during the dough mixing stage of the bread-making process. For example, in the case of sponge and dough process, the baking enzyme composition may be either added during mixing of the "sponge" dough or during mixing of the final dough. If added in at the sponge stage, there may be increased benefit in terms of functionality of the enzymes due to the additional preliminary fermentation time (typically 2 to 4 hours) that is available.

The baking enzyme composition in accordance with an embodiment of the invention begins functioning as soon as water is added to the dough during mixing. The rate of action of the enzymes is temperature dependent so that rate of action increases during final proof and early stages of baking until deactivation of the enzymes occurs during the latter stages of baking at temperatures of greater than, for example, 60 degrees Celsius.

The baking enzyme composition according to an embodiment of the invention may further comprise additional enzymes, flour or dough or bread agents or conditioners or additives, or the like. The additional enzyme may be of any origin, including plant, mammalian, and microbial (fungal, yeast, or bacterial) origin and may be obtained by techniques conventionally used in the industry.

In flour, dough and bread making, the baking enzyme composition in accordance with an embodiment of the invention may be used in combination with other flour, dough or bread ingredients or additives such as salt, oxidants (for example, ascorbic acid, and the like) emulsifiers (DATEM, SSL, CSL), enzymatic processing aids (for example, oxidoreductases, glucose oxidase, and the like), polysaccharide modifying enzymes (for example, α -amylase, hemicellulose, branching enzymes, and the like), protein modifying enzymes (for example, endoprotease, exoprotease, branching enzymes, and the like), or the like.

The term “flour” is defined herein as any milled or ground cereal grain including, but not limited to, wheat, barley, buckwheat, oat, maize, rice, triticale, oat, rye, and mixtures thereof.

The term “dough” is defined herein as any mixture of flour and other ingredients that can be kneaded or rolled into shape, and may be fresh, frozen, pre-pared, pre-baked, pre-steamed, pre-fried, or the like.

The term “effective amount” is defined herein as an amount of baking enzyme composition according to an embodiment of the invention that is enough or sufficient to result in activity having a measurable effect on at least one property of interest of the flour, dough, product resulting from the flour or dough, or the like.

The term “baked product” is defined herein as any product prepared from a flour and a dough, including but not limited bread, buns, rolls, baguette, pastries, roissants, pasta, noodles (stir-fried or boiled), tacos, tortillas, pita bread, cakes, pie crusts, muffins, pancakes, steamed bread, fried dough, doughnuts, crisp bread, cookies, biscuits, and the like.

The phrase “incorporating into the dough” is defined herein as adding the baking enzyme composition according to an embodiment of the invention to the dough, including any ingredient into the dough, at any time during the dough preparation and baking process, and may be added in one, two or more steps, which may be baked, fried, steamed, or other methods well known in the industry.

The term “improved property” is defined herein as any property of flour, dough, a product resulting from the flour or dough, in particular a baked or fried product, or the like, which is improved by the

action of the baking enzyme composition in accordance with an embodiment of the invention, relative to a flour, dough, baked product, or the like that the baking enzyme composition in accordance with an embodiment of the invention is not incorporated. The improved property may comprise, but is not limited to, improved elasticity, improved strength, improved mixing tolerance, improved water absorption, improved gas retention, improved volume, or the like of the flour, dough, a product resulting from the flour or dough, such as a baked product, or the like.

The term “improved elasticity” is defined herein as the property of dough which has a higher tendency to regain its original shape after being subjected to a certain physical stress or strain.

The term “improved strength” is defined herein as the property of dough which has a higher elasticity quality and requires more work to knead, mould or shape due to increased bond formations of the gluten matrix of the gluten strands formed by the gliadin and glutenin enzymes during dough processing.

The term “improved mixing tolerance” is defined herein as the property of dough which is less susceptible to physical alteration due to increased bond formations of the gluten matrix of the gluten strands formed by the gliadin and glutenin enzymes during dough processing and through the final proof stage of the baking process.

The term “improved water absorption” is defined herein as the property of dough to absorb water retention due to increased bond formations of the gluten matrix of the gluten strands formed by the gliadin and glutenin enzymes during dough processing. Water absorption is measured by taking farinogram readings.

The term “improved gas retention” is defined herein as the property of dough to retain gas due to increased bond formations of the gluten matrix of the gluten strands formed by the gliadin and glutenin enzymes during dough processing.

The term “improved volume” is defined herein as the volume of a baked product resulting from the baking process measured by analysis of the baked product with the rape seed displacement method, such as the volume of the baked good without the baked good tin divided by the mass of the same baked good measured by the rape seed displacement method. The unit for specific volume is millilitre per gram.

The term “pre-mix” is defined herein as a mix of baking enzyme composition in accordance with an embodiment of the invention with a suitable carrier such as flour, starch, a sugar, a complex

carbohydrate such as maltodextrin, a salt, and any additional baking aids including additives, flour agents, or dough conditioners.

When the baking enzyme composition according to an embodiment of the invention is incorporated in flour, dough, or a product resulting from the flour or dough, in an effective amount, several properties of the flour and dough, such as improved elasticity, improved strength, improved mixing tolerance, improved water absorption, improved gas retention, improved volume, or the like of the flour, dough, a product resulting from the flour or dough, such as a baked, fried, steamed, or the like product, or the like may be improved.

It has been surprisingly found that when the baking enzyme composition according to an embodiment of the invention has been added to a dough used to produce a baked product such as bread, a dough with improved elasticity, improved strength, improved mixing tolerance, improved water absorption, improved gas retention, improved volume, or the like, may be obtained while the baked product may show an improved bread volume.

The baking enzyme composition in accordance with embodiments of the invention may be in any suitable form, such as, in the form of a granulate, agglomerated powder or liquid, which enzyme compositions may be prepared by conventional methods well known in the industry. The baking enzyme composition may be prepared as a pre-mix or as part of a premix system that would be designed to be used at a dosage rate to suit the particular method of application, i.e., for production as a premix, the baking enzyme composition would be combined with suitable bulking agents to enable practical dosage rates. The pre-mix in accordance with an embodiment of the invention may comprise the enzymes as discussed herein, for example, lipases, oxido-reductases, xylanases and transferases. The pre-mix may also comprise a diluent, bulking agent, neutral carrier, or the like, such as flour, wheat gluten, starch mineral salts, wheat flour, or the like, or a combination of one or more of these. The enzymes of the pre-mix baking enzyme composition in accordance with an embodiment of the invention may be suitably diluted with a bulking agent to enable a practical dosage rate. A range of baked products based on the baking enzyme composition in accordance with an embodiment of the invention is envisaged.

The baking enzyme composition in accordance with embodiments of the invention relates to flour and dough (fresh or frozen), and baked or fried products resulting from the flours or doughs, based products used to prepare flour based consumer products, baked products, such as breads, baguettes, buns, rolls, pizzas crusts, pastry, pretzels, bagels, cakes, doughnuts, and the like.

Embodiments of the invention have been described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by the applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

Embodiments of the invention will be further elucidated by way of the following examples. The following examples incorporated herein and forming a part of the specification illustrate several aspects of the present invention and, together with the description, serve to explain the principles of the invention. While the invention will be described in connection with certain embodiments, there is no intent to limit the invention to those embodiments described. On the contrary, the intent is to cover all alternatives, modifications and equivalents as included within the scope of the invention as defined by the appended claims. In the examples:

EXAMPLE I

Baking trials have been conducted using different dosages of the baking enzyme composition in accordance with an embodiment of the invention versus different levels of added vital wheat gluten. The results of farinogram and extensogram readings are shown in the graphs of FIG. 3-5 and FIG. 6-8, respectively. The baking test results show the baking performance of the baking enzyme composition. Physical dough tests such as farinogram and extensogram measure the rheological properties of the dough. The effect of addition of 500ppm of a premix of the baking enzyme composition in accordance with an embodiment of the invention versus 1% added vital wheat gluten in an untreated bakers flour was shown with farinogram and extensogram of FARINOGRAPH and EXTENSOGRAF readings. The graphs of FIG. 3-5 diagrammatically show the farinogram curves produced via measuring and recording changes in torque developed by the action of the mixer blades during mixing of the dough. The graphs of FIG. 6-8 diagrammatically show the extensogram curves measured via uniaxial load extension for dough test pieces which have been subjected to controlled stretching to breaking point. A mixer that may be used for this purpose is a BRABENDER sigma-blade mixer. BRABENDER, FARINOGRAPH, and EXTENSOGRAF are registered trademarks of Brabender GmbH & Co. KG of Duisburg, Germany.

Sample 1 is control flour, Sample 2 is control flour plus 1% vital wheat gluten, and Sample 3 is control flour plus baking enzyme composition in accordance with an embodiment of the invention. During the farinogram, the BRABENDER mixer in operation was 300g having a speed of 63 1/minute.

The baking enzyme composition in accordance with an embodiment of the invention of Sample 3 comprises a premix baking enzyme composition added to the control flour. The activity units of the premix baking enzyme composition of the respective enzyme activities per gram of the baking enzyme composition based on the concentration of the activities in the premixed baking enzyme composition used during the bake tests, and used in obtaining the farinograms of FIG. 3-5 and extensograms of FIG. 6-8, as follows:

Glucose oxidase:	395 GOX U/g;
Lipid degrading enzymes:	85 PHOS/g;
Xylan degrading enzymes:	315 AXU/g; and
Transglutaminase:	1.16 TGU/g.

For the farinogram and extensogram readings, Sample 3 comprised 0.5g premixed baking enzyme composition/Kg of flour (500ppm) equivalent to the above concentration in terms of activity units/g of premixed baking enzyme composition. The premixed baking enzyme composition also comprised, in addition to the enzymes, a neutral carrier (wheat flour) in this embodiment to enable practical dosing and to ensure proper dispersion in the dough.

FIG. 3 is a graph showing a farinogram reading 40 relating to mixing of Sample 1 dough with control flour. The graph shows the top of the curve 42, middle of the curve 44, bottom of the curve 46 in relation to the 500 farinogram units (FU). The arrival time 48 is shown when the top curve 46 reaches the 500 FU line, and the departure time 50 is shown when the top curve 46 drops below the 500 FU line. The development time 52 is determined by the first addition of water and development of the dough's maximum consistency. The time between the arrival time 48 and the departure time 50 is the stability to give an indication of the flour's tolerance during mixing. The point of degree of softening 54 during mixing is measured 12 minutes after peak/development time 52. The farinogram shows the following:

Sample 1 – Control Flour – Farinogram Results

Moisture content:	14.0%
Consistency:	496 FU
Water absorption:	62.5%
Water absorption (corrected for 500 FU):	62.4%

Water absorption (corrected to 14.0%)	62.4%
Development time:	7.5 minutes
Stability:	13.8 minutes
Degree of softening (10 minutes after begin):	11 FU
Degree of softening (ICC/12 minutes after max):	38 FU
Farinogram quality number:	148

FIG. 4 is a graph showing a farinogram reading 60 of mixing of Sample 2 dough with control flour and vital wheat gluten. The graph shows the top of the curve 62, middle of the curve 64, bottom of the curve 66 in relation to the 500 farinogram units (FU) for reading the arrival time 68 and the departure time 70 to determine the stability, development time 72 and degree of softening 74 during mixing.

Sample 2 – Control Flour plus 1% Vital Wheat Gluten – Farinogram Results

Moisture content:	14.0%
Consistency:	502 FU
Water absorption:	64.3%
Water absorption (corrected for 500 FU):	64.4%
Water absorption (corrected to 14.0%)	64.4%
Development time:	7.8 minutes
Stability:	14.9 minutes
Degree of softening (10 minutes after begin):	6 FU
Degree of softening (ICC/12 minutes after max):	35 FU
Farinogram quality number:	164

FIG. 5 is a graph showing a farinogram reading 80 relating to the mixing of Sample 3 dough with control flour and baking enzyme composition in accordance with an embodiment of the invention. The graph shows the top of the curve 82, middle of the curve 84, bottom of the curve 86 in relation to the 500 farinogram units (FU) for reading the arrival time 88 and the departure time 90 to determine the stability and degree of softening (which is zero) and development time 92 during mixing.

Sample 3 – Control Flour plus Baking Enzyme Composition – Farinogram Results

Moisture content:	14.0%
Consistency:	502 FU
Water absorption:	63.9%

Water absorption (corrected for 500 FU):	64.0%
Water absorption (corrected to 14.0%)	64.0%
Development time:	20.1 minutes
Stability:	24.8 minutes
Degree of softening (10 minutes after begin):	0 FU
Degree of softening (ICC/12 minutes after max):	0 FU
Farinogram quality number:	300

FIG. 6 is a graph showing an extensogram reading 100 relating to the mixing of Sample 1 dough with control flour. The extensogram shows the extensibility for determining the elasticity or stretching behaviour of the dough of the Sample 1 dough with a first curve 102 of Test 2, second curve 104 of Test 1, and 5cm point 106. The area below the curves is an indication of the energy, and the resistance/extensibility is the ratio number taken at the 5cm point.

Sample 1 – Control Flour – Extensogram Results

Test after 45 Minutes Water absorption:	59.7%		
	Test 1	Test 2	Mean Value
Energy [cm ²]:	138	125	131
Resistance to Extension [BU]:	460	421	440
Extensibility [mm]:	163	159	161
Maximum [BU]:	631	572	602
Ratio Number:	2.8	2.7	2.7
Ratio Number (Max):	3.9	3.6	3.7

FIG. 7 is a graph showing a extensogram reading relating to the mixing of Sample 2 dough with control flour and vital wheat gluten. The extensogram shows the extensibility of the Sample 2 dough with a first curve 112 of Test 2, second curve 114 of Test 1, and 5cm point 116.

Sample 2 – Control Flour plus 1% Vital Wheat Gluten – Extensogram Results

Test after 45 Minutes Water absorption:	61.6%		
	Test 1	Test 2	Mean Value
Energy [cm ²]:	116	119	117
Resistance to Extension [BU]:	400	371	386
Extensibility [mm]:	150	164	157
Maximum [BU]:	590	541	566
Ratio Number:	2.7	2.3	2.5

Ratio Number (Max):	3.9	3.3	3.6
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FIG. 8 is a graph showing a extensogram reading 120 relating to the mixing of Sample 3 dough with control flour and baking enzyme composition in accordance with an embodiment of the invention. The extensogram shows the extensibility of the Sample 2 dough with a first curve 122 of Test 1, second curve 124 of Test 2, and 5cm point 126.

Sample 3 – Control Flour plus Baking Enzyme Composition – Extensogram Results

Test after 45 Minutes water absorption: 61.8%

	Test 1	Test 2	Mean Value
Energy [cm ²]:	122	129	125
Resistance to Extension [BU]:	358	371	364
Extensibility [mm]:	168	172	170
Maximum [BU]:	540	558	549
Ratio Number:	2.1	2.2	2.1
Ratio Number (Max):	3.2	3.2	3.2

The farinogram of Sample 3, which has an addition of 500ppm of the premix of baking enzyme composition in accordance with an embodiment of the invention, shows a significant increase in dough mixing tolerance (stability) compared to the control flour (Sample 1) and also compared to the flour sample with 1% added vital wheat gluten (Sample 2). It is also confirmed that farinogram dough water absorption with addition of 500ppm premix of baking enzyme composition (64.0%) is significant higher compared to that of the control flour (59.6%) and is not significantly different to the water absorption of Sample 2 (64.4%), which is the sample with 1% added vita wheat gluten.

The extensogram test results for determining the elasticity or stretching behaviour of the dough have not shown any significant difference in resistance to extension between Sample2 (1% addition of gluten) and Sample 3 (500ppm premix of baking enzyme composition). Sample 3 which has 500ppm of baking enzyme composition has, however, a slightly higher extensibility (170mm) compared to Sample 2 with 1% gluten (157 mm) and that of the control flour (161mm) of Sample 1.

EXAMPLE II

Bake tests were carried using the sponge and dough method of bread-making with a typical white sandwich bread formulation based on a general-purpose type flour of 10.5% protein content ('control flour') instead of what would be generally regarded in the industry as a good bakers' flour,

which would typically have a protein content of 11.5% to 12.5%. In the baking tests of EXAMPLE II, 0.5 g premix baking enzyme composition/Kg flour (500ppm), 0.75 g premix baking enzyme composition/Kg flour (750 ppm), and 1 g premix baking enzyme composition/Kg flour (1000ppm) was used and equivalent to in terms of activity units/g of premixed baking enzyme composition as used in Sample 3 of EXAMPLE I.

Various dosages (1%, 2% and 4%), T1, T2, T3, respectively, based on total flour weight of vital wheat gluten were added at the final mixing stage and compared to a corresponding series of tests with addition of various levels (500ppm, 750ppm and 1000ppm), T4, T5, T6, respectively, based on total flour weight of a premix of the baking enzyme composition. Where T1 = control flour + 1% vital wheat gluten; T2 = control flour + 2% vital wheat gluten; T3 = control flour + 4% vital wheat gluten; T4 = control flour + 500ppm premix – baking enzyme composition; T5 = control flour + 750ppm baking enzyme composition; and T6 = control flour + 1000ppm premix – baking enzyme composition.

Total dough water absorption:

T1: 58.0%	T2: 59.0%	T3: 62.0%	T4: 58.0%	T5: 59.0%	T6: 62.0%
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No significant differences in dough consistency or dough tackiness were observed during dough processing.

FIG. 9-11 demonstrate the effectiveness of the baking enzyme composition in accordance with an embodiment of the invention as an enhancer of the endogenous gluten protein of the control flour versus supplementation of the control flour's endogenous protein with vital wheat gluten. The baking enzyme composition promotes higher loaf volume than conventional vital wheat gluten.

External:

FIG. 9 shows the external baked product results 130 relating to a comparison of baked products without (T1 132, T2 134, T3 136) and baked products with (T4 138, T5 140, T6 142) the baking enzyme composition in accordance with an embodiment of the invention. The specific volumes are:

Specific volumes:

T1: 5.60	T2: 5.75	T3: 5.77	T4: 6.07	T5: 6.20	T6: 6.33
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FIG. 10 shows the internal baked products of FIG. 8, and the results relating to a comparison of baked product without (T1 152, 154; T2 156, 158; T3 160, 162) and baked products with (T4 164, 166; T5 168, 170; T6 172, 174) the baking enzyme composition in accordance with an embodiment of the

invention. The internal structure shows no significant difference observed in crumb structure for T1 versus T4; T2 versus T5; and T3 versus T6.

The specific volume for each sample T1-T6 results:

Test	Specific Volume (ml/g)	Vital Wheat Gluten (%)	Baking Enzyme Composition (ppm)
T1	5.6	1.00	
T2	5.75	2.00	
T3	5.77	4.00	
T4	6.07		500
T5	6.20		750
T6	6.33		1000

FIG. 11 is a graph 180 showing the results of the specific volume versus addition of vital wheat gluten curve 182 and the baking enzyme composition curve 184.

EXAMPLE III

FIG. 12 shows the external baked product results 200 of doughs shocked by drop test after final proof relating to a comparison of baked products without and baked products with the baking enzyme composition in accordance with an embodiment of the invention. Like in Example II, T1 = control flour + 1% vital wheat gluten 202; T2 = control flour + 2% vital wheat gluten 204; T3 = control flour + 4% vital wheat gluten 206; T4 = control flour + 500ppm premix – baking enzyme composition 208; T5 = control flour + 750ppm baking enzyme composition 210; and T6 = control flour + 1000ppm premix – baking enzyme composition 212.

Specific volumes:

T1: 4.15 T2: 4.16 T3: 4.55 T4: 5.16 T5: 5.62 T6: 5.73

Doughs with added baking enzyme composition (as seen in T4, T5 and T6) show higher levels of dough shock tolerance than the respective doughs prepared with added vital wheat gluten (T1, T2 & T3)

CLAIMS:

1. A baking enzyme composition for flour comprising a lipase, an oxido-reductase, a xylanase and a transferase, wherein the lipase is between 5 to 200 units per kilogram of flour; the oxido-reductase is between 5 to 1000 units per kilogram of flour; the xylanase is between 10 to 1000 units per kilogram of flour; and the transferase is between 0.1 to 5 units per kilogram of flour.
2. The baking enzyme composition of claim 1 wherein the baking enzyme composition is derived from microbial origin.
3. The baking enzyme composition of claim 1 wherein the lipase is derived from *Aspergillus niger*, *Bacillus subtilis*, *Aspergillus oryzae*, *Rhizopus oryzae*, or *Fusarium oxysporum*.
4. The baking enzyme composition of claim 1 wherein the oxido-reductase is glucose oxidase.
5. The baking enzyme composition of claim 1 wherein the oxido-reductase is derived from *Aspergillus niger*.
6. The baking enzyme composition of claim 1 wherein the xylanase is fungal xylanase or beta-glucanase.
7. The baking enzyme composition of claim 1 wherein the xylanase is derived from *Aspergillus oryzae* or *Aspergillus niger*.
8. The baking enzyme composition of claim 1 wherein the transferase is transglutaminase.
9. The baking enzyme composition of claim 1 wherein the transferase is derived from *Aspergillus niger* or *Streptomyces mobaraensis*.
10. The baking enzyme composition of claim 1 wherein the lipase is between 15 to 90 units per kilogram of flour; the oxido-reductase is between 70 to 420 units per kilogram of flour; the xylanase is between 55 to 330 units per kilogram of flour; and the transferase is between 0.1 to 1.2 units per kilogram of flour.
11. A pre-mix comprising a flour having an endogenous gluten forming protein; and the baking enzyme composition according to any one of claims 1 to 10.
12. A dough comprising a baking enzyme composition according to any one of claims 1 to 10.

13. A method to prepare a dough comprising adding to dough comprising flour, water, yeast, and the baking enzyme composition or pre-mix according to any one of claims 1 to 10.
14. A method of enhancing the endogenous vital gluten content of a flour comprising adding the baking enzyme composition according to any one of claims 1 to 10.
15. A food product comprising the baking enzyme composition according to any one of claims 1 to 10.