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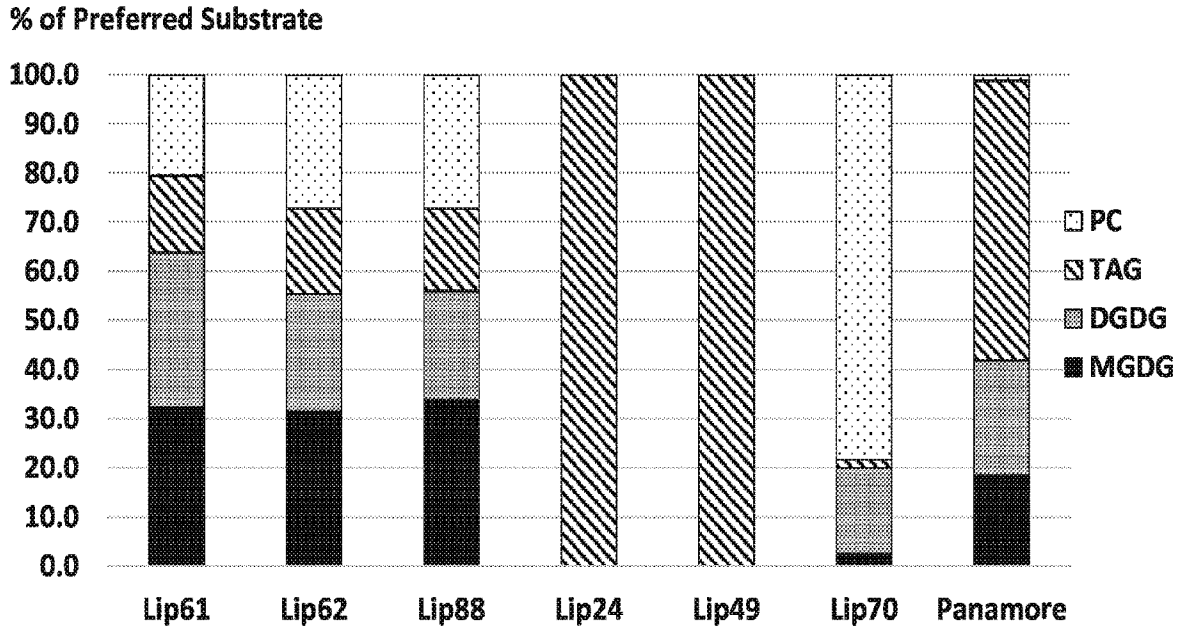

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Lipase enzymes and methods of using the lipases in a baking for improving the volume, stability, tolerance of a baked product and/or reducing and reducing or eliminating the use of DATEM.

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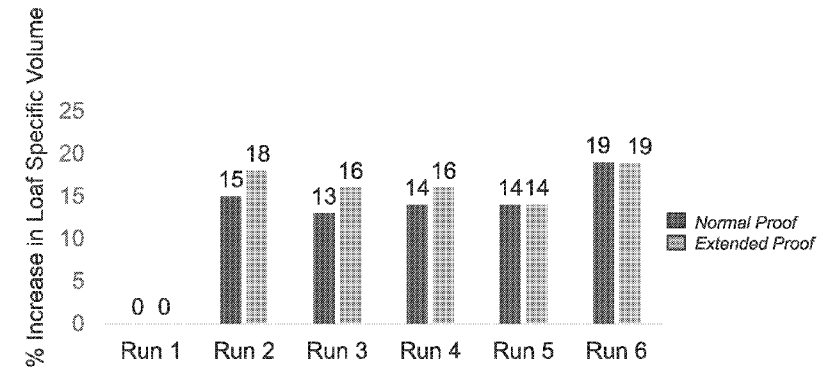

DATEM	0	100	0	3	6	13
Lip62	0	0	100	100	100	100

(% dosage)

Figure 2

(57) Abstract: Lipase enzymes and methods of using the lipases in a baking for improving the volume, stability, tolerance of a baked product and/or reducing and reducing or eliminating the use of DATEM.

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BAKING LIPASES

SEQUENCE LISTING

This application includes an amino acid sequence listing in computer readable form (CRF) in an ASC II text (.txt) file as identified below and is hereby incorporated by reference into the specification of this application in its entirety and for all purposes.

File Name	Date of Creation	Size (bytes)
150300_SequenceListing	January 26, 2016	26.3 KB (27,009 bytes)

TECHNICAL FIELD

Bread has been a staple of human nutrition for thousands of years. Bread is usually made by combining a flour, water, salt, yeast, and/or other food additives to make a dough or paste; then the dough is baked to make bread. Enzymes are known to be useful in baking because of the enzymes effects on the baking process can be similar or better than chemical alternatives. Several different enzymes can be used for making bread, for example lipases have been known to improve the stability and volume of the bread; however, the industry still needs a lipase that improves volume, stability, tolerance, reduces or eliminates the additive diacetyl tartaric acid esters of monoglycerides (DATEM). This disclosure is directed to a lipase that meets or exceeds these industrial requirements.

BRIEF SUMMARY OF THE INVENTION

An embodiment of the invention is: A method for increasing the volume of a baked product comprising: (a) providing a dough; (b) providing a lipase, wherein the lipase is a polypeptide having the amino acid sequence of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11; (c) combining the lipase of (b) with the dough of (a) and baking the combination to generate the baked product having an increased volume.

In another embodiment, the dough is a composition comprising: a flour, a salt, water, and yeast.

In another embodiment, the flour is selected from the group consisting of: almond flour, coconut flour, chia flour, corn flour, barley flour, spelt flour, soya flour, hemp flour, potato flour, quinoa, teff flour, rye flour, amaranth flour, arrowroot flour, chick pea (garbanzo) flour, cashew flour, flax meal, macadamia flour, millet flour, sorghum flour, rice flour, tapioca flour, and any combination thereof.

In another embodiment, the yeast is selected from the group consisting of: bakers' yeast, cream yeast, compressed yeast, cake yeast, active dry yeast, instant yeast, osmotolerant yeasts, rapid-rise yeast, deactivated yeast, nutritional yeast, brewer's yeast, distiller's and wine yeast.

In another embodiment, the lipase is a variant polypeptide and the variant polypeptide is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the an amino acid sequence selected from the group consisting of: the polypeptide as set forth in the amino acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, and the variant polypeptide has lipase activity.

In another embodiment, the lipase is a polypeptide encoded by a nucleic acid sequence that encodes that amino acid sequence selected from the group consisting of: a nucleic acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12.

In another embodiment, further comprising the addition of a second enzyme. In a further embodiment, the second enzyme comprisies a second lipase, an Alpha-amylase; a Glucan 1, 4-alpha-maltotetraohydrolase; an exo-maltotetraohydrolase; a G4-amylase; a Glucan 1,4-alpha-maltohydrolase; a maltogenic alpha-amylase; a cyclodextrin glucanotransferase; a CGTase; a glucoamylase; an Endo-1,4-beta-xylanase; a xylanase; a cellulase; an Oxidoreductases; a Phospholipase A1; a Phospholipase A2; a Phospholipase C; a Phospholipase D; a Galactolipase, triacylglycerol lipase, an arabinofuranosidase, a transglutaminase, a pectinase, a pectate lyase, a protease, or any combination thereof.

In another embodiment, the lipase is active at a range from pH 4.0 to pH 12.0.

In another embodiment, the lipase is active at a temperature range from 20 ° C to 60° C.

In another embodiment of the invention, is method for increasing the volume of a baked product without the addition of DATEM comprising: (a) providing a dough; (b) providing a lipase; , wherein the lipase is a polypeptide having the amino acid sequence of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11, (c) combining the lipase of (b) with the dough of (a) without the addition of DATEM and baking the combination to generate the baked product having an increased volume.

In another embodiment, the dough is a composition comprising: a flour, a salt, water, and yeast.

In another embodiment, the flour is selected from the group consisting of: almond flour, coconut flour, chia flour, corn flour, barley flour, spelt flour, soya flour, hemp flour, potato flour, quinoa, teff flour, rye flour, amaranth flour, arrowroot flour, chick pea (garbanzo) flour, cashew

flour, flax meal, macadamia flour, millet flour, sorghum flour, rice flour, tapioca flour, and any combination thereof.

In another embodiment, the yeast is selected from the group consisting of: bakers' yeast, cream yeast, compressed yeast, cake yeast, active dry yeast, instant yeast, osmotolerant yeasts, rapid-rise yeast, deactivated yeast, nutritional yeast, brewer's yeast, distiller's and wine yeast.

In another embodiment, the lipase is a polypeptide having an amino acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11.

In another embodiment, the lipase is a variant polypeptide and the variant polypeptide is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the an amino acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, and the variant polypeptide has lipase activity.

In another embodiment, the lipase is a polypeptide encoded by a nucleic acid sequence that encodes that amino acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11.

In another embodiment, the method further comprising the addition of a second enzyme. In a further embodiment, the second enzyme comprisies a second lipase, an Alpha-amylase; a Glucan 1, 4-alpha-maltotetraohydrolase; an exo-maltotetraohydrolase; a G4-amylase; a Glucan 1,4-alpha-maltohydrolase; a maltogenic alpha-amylase; a cyclodextrin glucanotransferase; a CGTase; a glucoamylase; an Endo-1,4-beta-xylanase; a xylanase; a cellulase; an Oxidoreductases; a Phospholipase A1; a Phospholipase A2; a Phospholipase C; a Phospholipase D; a Galactolipase, triacylglycerol lipase, an arabinofuranosidase, a transglutaminase, a pectinase, a pectate lyase, a protease, or any combination thereof.

In another embodiment, the lipase is active at a range from pH 4.0 to pH 12.0.

In another embodiment, the lipase is active at a temperature range from 20 ° C to 60° C.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING

Figure 1. Lipase specificity for natural substrates in solution.

Figure 2. Lipase and DATEM dosage with Pistolet test

DETAILED DESCRIPTION OF THE INVENTION

Bread includes, but is not limited to: rolls, buns, pastries, cakes, flatbreads, pizza bread, pita bread, wafers, pie crusts naan, lavish, pitta, focaccia, sourdoughs, noodles, cookies, tortillas, pancakes, crepes, croutons, and biscuits. Baking bread generally involves mixing ingredients to

form dough, kneading, rising, shaping, baking, cooling and storage. The ingredients used for making dough generally include flour, water, salt, yeast, and other food additives.

Flour is generally made from wheat and can be milled for different purposes such as making bread, pastries, cakes, biscuits pasta, and noodles. Alternatives to wheat flour include, but are not limited to: almond flour, coconut flour, chia flour, corn flour, barley flour, spelt flour, soya flour, hemp flour, potato flour, quinoa, teff flour, rye flour, amaranth flour, arrowroot flour, chick pea (garbanzo) flour, cashew flour, flax meal, macadamia flour, millet flour, sorghum flour, rice flour, tapioca flour, and any combination thereof. Flour type is known to vary between different regions and different countries around the world.

Yeast breaks down sugars into carbon dioxide and water. A variety of Baker's yeast, which are usually derived from *Saccharomyces cerevisiae,* are known to those skilled in the art including, but not limited to: cream yeast, compressed yeast, cake yeast, active dry yeast, instant yeast, osmotolerant yeasts, rapid-rise yeast, deactivated yeast. Other kinds of yeast include nutritional yeast, brewer's yeast, distiller's and wine yeast.

Sweeteners include but are not limited to: liquid sugar, syrups, white (granulated) sugars, brown (raw) sugars, honey, fructose, dextrose, glucose, high fructose corn syrup, molasses, and artificial sweeteners

Emulsifiers include but are not limited to diacetyl tartaric acid esters of monoglycerides (DATEM), sodium stearoyl lactylate (SSL), calcium stearoyl lactylate (CSL), ethoxylated mono- and diglycerides (EMG), polysorbates (PS), and succinylated monoglycerides (SMG).

Other food additives that can be used with the methods of this disclosure include: Lipids, oils, butter, margarine, shortening, butterfat, glycerol, eggs, diary, non-diary alternatives, thickeners, preservatives, colorants, and enzymes.

An enzyme is a biological molecule comprising a sequence of amino acids, wherein the enzyme can catalyze a reaction. Enzyme names are known to those skilled in the art based on the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB). Enzyme names include: an EC (Enzyme Commission) number, recommended name, alternative names (if any), catalytic activity, and other factors. Enzymes are also known as a polypeptide, a protein, a peptide, an amino acid sequence, or is identified by a SEQ ID NO. In this disclosure, the alternative names for enzyme can be used interchangeably.

Different classes of enzymes are known to be useful in baking, including: Alpha-amylase (E.C. 3.2.1.1); Glucan 1, 4-alpha-maltotetraohydrolase (E.C. 3.2.1.60), also known as exo-maltotetraohydrolase, G4-amylase; Glucan 1,4-alpha-maltohydrolase (E.C. 3.2.1.133), also

known as maltogenic alpha-amylase; Endo-1,4-beta-xylanase (E.C. 3.2.1.8); Oxidoreductases; Phospholipase A1 (E.C. 3.1.1.32) Phospholipase A2 (E.C. 3.1.1.4); Phospholipase C (E.C. 3.1.4.3); Phospholipase D (E.C. 3.1.4.4); Galactolipase (E.C. 3.1.1.26), and Protease. Enzymes are used as food ingredients, food additives, and/processing aids.

Lipases (E.C. 3.1.1.3) are hydrolytic enzymes that are known to cleave ester bonds in lipids. Lipases include phospholipases, triacylglycerol lipases, and galactolipases. Lipases have been identified from plants; mammals; and microorganisms including but not limited to: *Pseudomonas, Vibrio, Acinetobacter, Burkholderia, Chromobacterium,* Cutinase from *Fusarium solani* (FSC), *Candida antarctica A* (CalA), *Rhizopus oryzae* (ROL), *Thermomyces lanuginosus* (TLL) *Rhizomucor miehei* (RML), *Aspergillus Niger, Fusarium heterosporum, Fusarium oxysporum, Fusarium culmorum* lipases.

In addition, many lipases, phospholipases, and galactolipases have been disclosed in patents and published patent applications including, but not limited to: WO1993/000924, WO2003/035878, WO2003/089620, WO2005/032496, WO2005/086900, WO2006/031699, WO2008/036863, and WO2011/046812.

Commercial lipases used in food processing and baking including, but not limited to: LIPOPAN™, NOOPAZYME, (available from Novozymes); PANAMORE, CAKEZYME, and BAKEZYME (available from DSM); and GRINDAMYL EXEL 16, GRINDAMYL POWERBAKE, and TS-E 861 (available from Dupont/Danisco).

A lipase of this disclosure is an isolated, synthetic, or recombinant polypeptide as set forth in the amino acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11.

A lipase of the disclosure is an isolated, synthetic, or recombinant polypeptide encoded by a polynucleotide as set forth in the nucleic acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12.

The lipase having an amino acid sequence of SEQ ID NO:1 is encoded by the polynucleotide having a nucleic acid sequence of SEQ ID NO:2, or a polynucleotide variant of SEQ ID NO:2 that encodes the amino acid sequence of SEQ ID NO:1. The lipase having an amino acid sequence of SEQ ID NO:3 is encoded by the polynucleotide having a nucleic acid sequence of SEQ ID NO:4, or a variant of SEQ ID NO:4 that encodes the amino acid sequence of SEQ ID NO:3. The lipase having an amino acid sequence of SEQ ID NO:5 is encoded by the polynucleotide having a nucleic acid sequence of SEQ ID NO:6, or a variant of SEQ ID NO:6 that encodes the amino acid sequence of SEQ ID NO:5. The lipase having an amino acid sequence of SEQ ID NO:7 is encoded

by the polynucleotide having a nucleic acid sequence of SEQ ID NO:8, or a variant of SEQ ID NO:8 that encodes the amino acid sequence of SEQ ID NO:7. The lipase having an amino acid sequence of SEQ ID NO:9 is encoded by the polynucleotide having a nucleic acid sequence of SEQ ID NO:10, or a variant of SEQ ID NO:10 that encodes the amino acid sequence of SEQ ID NO:9. The lipase having an amino acid sequence of SEQ ID NO:11 is encoded by the polynucleotide having a nucleic acid sequence of SEQ ID NO:12, or a variant of SEQ ID NO:12 that encodes the amino acid sequence of SEQ ID NO:11.

A lipase of this disclosure is an isolated, synthetic, or recombinant variant polypeptide comprising an amino acid sequence that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the full length enzymatically active polypeptide of the amino acid sequence comprising or selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11, and wherein the variant polypeptide has lipase activity.

A lipase of this disclosure, is an isolated, synthetic, or recombinant variant polypeptide comprising an enzymatically active polypeptide of the amino acid sequence comprising or selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11 and an amino acid substitution, an amino acid insertion, an amino acid deletion, or any combination thereof, wherein the variant polypeptide has lipase activity.

In a further embodiment, the variant polypeptide having an amino acid substitution can be a conservative amino acid substitution. A "conservative amino acid substitution" means replacement of one amino acid residue in an amino acid sequence with a different amino acid residue having a similar property at the same position compared to the parent amino acid sequence. Some examples of a conservative amino acid substitution include but are not limited to replacing a positively charged amino acid residue with a different positively charged amino acid residue; replacing a polar amino acid residue with a different polar amino acid residue; replacing a non-polar amino acid residue with a different non-polar amino acid residue, replacing a basic amino acid residue with a different basic amino acid residue, or replacing an aromatic amino acid residue with a different aromatic amino acid residue.

In a further embodiment, the variant polypeptide having an amino acid substitution can be a replacement of one amino acid residue for any other amino acid residue, wherein the variant polypeptide has lipase activity.

In a further embodiment, the variant polypeptide having lipase activity is a "mature polypeptide." A mature polypeptide means an enzyme in its final form including any post-

translational modifications, glycosylation, phosphorylation, truncation, N-terminal modifications, C-terminal modifications, signal sequence deletion. A mature polypeptide can vary depending upon the expression system, vector, promoter, and/or production process.

In a further embodiment, a lipase is active over a broad pH at any single point within the range from about pH 4.0 to about pH 12.0. In an embodiment, the lipase is active over a range of pH 4.0 to pH 11.0, pH 4.0 to pH 10.0, pH 4.0 to pH 9.0, pH 4.0 to pH 8.0, pH 4.0 to pH 7.0, pH 4.0 to pH 6.0, or pH 4.0 to pH 5.0. In another embodiment the lipase is active at pH 4.0, pH 4.5, pH 5.0, pH 5.5, pH 6.0, pH 6.5, pH 7.0, pH 7.5, pH 8.0, pH 8.5, pH 9.0, pH 9.5, pH 10.0, pH 10.5, pH 11.0, pH 11.5, pH 12.0, and pH 12.5.

In a further embodiment, a lipase is active over a broad temperature used in at any time during a baking process, wherein the temperature is any point in the range from about 20 °C to about 60°C. In another embodiment, the lipase is active at a temperature range from 20 ° C to 55 ° C, 20 ° C to 50 ° C, 20 ° C to 45 ° C, 20 ° C to 40 ° C, 20 ° C to 35 ° C, 20 ° C to 30 ° C, or 20 ° C to 25° C. In another embodiment the lipase is active at a temperature of at least 19 ° C, 20 ° C, 21 ° C, 22 ° C, 23 ° C, 24 ° C, 25 ° C, 26 ° C, 27 ° C, 28 ° C, 29 ° C, 30 ° C, 31 ° C, 32 ° C, 33 ° C, 34 ° C, 35 ° C, 36 ° C, 37 ° C, 38 ° C, 39 ° C, 40 ° C, 41 ° C, 42 ° C, 43 ° C, 44 ° C, 45 ° C, 46 ° C, 47 ° C, 48 ° C, 49 ° C, 50 ° C, 51 ° C, 52 ° C, 53 ° C, 54 ° C, 55 ° C, 56 ° C, 57 ° C, 58 ° C, 59 ° C, 60 ° C, 61 ° C, 62 ° C, or higher temperatures. "Sequence Identity" means a comparison of a first amino acid sequence to a second amino acid sequence, or a comparison of a first nucleic acid sequence to a second nucleic acid sequence and is calculated as a percentage based on the comparison.

Generally, the created alignment can be used to calculate the sequence identity by one of two different approaches. In the first approach, both, mismatches at a single position and gaps at a single position are counted as non-identical positions in final sequence identity calculation. In the second approach, mismatches at a single position are counted as non-identical positions in final sequence identity calculation; however, gaps at a single position are not counted (ignored) as non-identical positions in final sequence identity calculation. In other words, in the second approach gaps are ignored in final sequence identity calculation. The differences between these two approaches, counting gaps as non-identical positions vs ignoring gaps, at a single position can lead to variability in sequence identity value between two sequences.

In an embodiment of this disclosure, sequence identity is determined by a program, which produces an alignment, and calculates identity counting both mismatches at a single position and gaps at a single position as non-identical positions in final sequence identity calculation. For example program Needle (EMBOS), which has implemented the algorithm of Needleman and

Wunsch (Needleman and Wunsch, 1970, J. Mol. Biol. 48: 443-453), and which calculates sequence identity by first producing an alignment between a first sequence and a second sequence, then counting the number of identical positions over the length of the alignment, then dividing the number of identical residues by the length of an alignment, then multiplying this number by 100 to generate the % sequence identity [% sequence identity = (# of Identical residues / length of alignment) x 100)].

In another embodiment of this disclosure, sequence identity can be calculated from a pairwise alignment showing both sequences over the full length, so showing the first sequence and the second sequence in their full length ("Global sequence identity"). For example program Needle (EMBOSS) produces such alignments; % sequence identity = (# of Identical residues / length of alignment) x 100)].

In another embodiment of this disclosure, sequence identity can be calculated from a pairwise alignment showing only a local region of the first sequence or the second sequence ("Local Identity"). For example program Blast (NCBI) produces such alignments; % sequence identity = (# of Identical residues / length of alignment) x 100)].

In an embodiment of the disclosure, the lipase can be used in combination with at least one other enzyme or a second enzyme. In another embodiment, the second enzyme comprises or is selected from the group consisting of: an Alpha-amylase; a Glucan 1, 4-alpha-maltotetraohydrolase, also known as exo-maltotetraohydrolase, G4-amylase; a Glucan 1,4-alpha-maltohydrolase, also known as maltogenic alpha-amylase, a cyclodextrin glucanotransferase, a glucoamylase; an Endo-1,4-beta-xylanase; a xylanase, a cellulase, an Oxidoreductases; a Phospholipase A1; a Phospholipase A2; a Phospholipase C; a Phospholipase D; a Galactolipase, triacylglycerol lipase, an arabinofuranosidase, a transglutaminase, a pectinase, a pectate lyase, a a protease, or any combination thereof. In another embodiment, the enzyme combination is the lipase disclosed herein and a maltogenic alpha-amylase, or the enzyme combination is the lipase disclosed herein, a maltogenic alpha-amylase, and a xylanase.

In another embodiment of the disclosure, the lipase can be a hybrid of more than one lipase enzymes. A "hybrid" or "chimeric" or "fusion protein" means that a domain of a first lipase of the disclosure is combined with a domain of a second lipase to form a hybrid lipase and the hybrid has lipase activity. In one embodiment a domain of a lipase of this disclosure is combined with a domain of a commercially available lipase, such as LIPOPAN (available from Novozymes), or PANAMORE (available from DSM) to form a hybrid lipase and the hybrid has lipase activity.

Industrial enzymes are usually recombinant proteins produced using bacteria, fungi, or yeast expression systems. "Expression system" also means a host microorganism, expression hosts, host cell, production organism, or production strain and each of these terms can be used interchangeably for this disclosure. Examples of expression systems include but are not limited to: *Aspergillus niger, Aspergillus oryzae, Hansenula polymorpha, Thermomyces lanuginosus, fusarium oxysporum, Fusarium heterosporum, Escherichia coli, Bacillus,* preferably *Bacillus subtilis,* or *Bacillus licheniformis, Pseudomonas,* preferably *Pseudomonas fluorescens, Pichia pastoris (also known as Komagataella phaffii), Myceliopthora thermophile (C1), Schizosaccharomyces pombe, Trichoderma,* preferably *Trichoderma reesei.* In an embodiment the lipase of this disclosure is produced using the expression system listed above.

Lipases are known to be useful for other industrial applications. In an embodiment of this disclosure, the lipase is used in a detergent. In an embodiment of this disclosure, the lipase is used in personal care products such as contact lens solution. In another embodiment, the lipase of this disclosure is used in the processing of textiles such as leather manufacturing. In another embodiment, the lipase of this disclosure can be used in pulp and paper processing. In a further embodiment, the pulp and paper processing is pitch control, or deinking. In another embodiment, a lipase of this disclosure can be used for manufacturing biodiesel. In another embodiment, a lipase of this disclosure can be used for cheese ripening. In another embodiment, lipases of this disclosure can be used in preparing a meat flavor and/or aroma. In another embodiment, a lipase of this disclosure can be used in the modification of oils & fats. In another embodiment, a lipase of this disclosure can be used in enzymatic oil degumming. In another embodiment, a lipase of this disclosure can be used in the production of ethanol.

The term "baked products" as used herein includes baked products such as bread, crispy rolls, sandwich bread, buns, baguette, ciabatta, croissants, as well as fine bakery wares like donuts, brioche, stollen, cakes, muffins, etc.

The term "dough" as used herein is defined as a mixture of flour, salt, yeast and water, which can be kneaded, molded, shaped or rolled prior to baking. In addition also other ingredients such as sugar, margarine, egg, milk, etc. might be used. The term includes doughs used for the preparation of baked goods, such as bread, rolls, sandwich bread, baguette, ciabatta, croissants, sweet yeast doughs, etc.

The term "bread volume" as used herein is the volume of a baked good determined by using a laser scanner (e.g. Volscan Profiler ex Micro Stable System) to measure the volume as well as the specific volume. The term also includes the volume which is determined by measuring the length, the width and the height of certain baked goods.

The term "comprising" as used herein is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps.

It is understood that aspects and embodiments of the invention described herein include "consisting" and/or "consisting essentially of" aspects and embodiments.

Throughout this disclosure, various aspects are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Other objects, advantages and features of the present disclosure will become apparent from the following specification taken in conjunction with the accompanying drawings.

In the following description, numerous specific details are set forth to provide a more thorough understanding of the present disclosure. However, it will be apparent to one of skill in the art that the methods of the present disclosure may be practiced without one or more of these specific details. In other instances, well-known features and procedures well known to those skilled in the art have not been described in order to avoid obscuring the disclosure.

The following embodiments are provided:

1. A method for increasing the volume of a baked product comprising:

(a) providing a dough;

(b) providing a lipase, wherein the lipase has lipase activity and comprises an amino acid sequence with at least 90% sequence identity to : SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11; and

(c) combining the lipase of (b) with the dough of (a) and baking the combination to generate the baked product having an increased volume.

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2. The method of embodiment 1, wherein the dough is a composition comprising: a flour, a salt, water, and yeast.

3. The method of embodiment 2, wherein the flour is selected from the group consisting of: almond flour, coconut flour, chia flour, corn flour, barley flour, spelt flour, soya flour, hemp flour, potato flour, quinoa, teff flour, rye flour, amaranth flour, arrowroot flour, chick pea (garbanzo) flour, cashew flour, flax meal, macadamia flour, millet flour, sorghum flour, rice flour, tapioca flour, and any combination thereof.

4. The method of embodiment 2, wherein the yeast is selected from the group consisting of: baker's yeast, cream yeast, compressed yeast, cake yeast, active dry yeast, instant yeast, osmotolerant yeasts, rapid-rise yeast, deactivated yeast, nutritional yeast, brewer's yeast, distiller's and wine yeast.

5. The method of embodiment 1, wherein the lipase has lipase activity and is at least 97% identical to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11.

6. The method of embodiment 1, wherein the lipase is a polypeptide encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12.

7. The method of embodiment 1, further comprising the addition of a second enzyme.

8. The method of embodiment 7, wherein the second enzyme comprises a second lipase, an Alpha-amylase; a Glucan 1, 4-alpha-maltotetraohydrolase; an exo-maltotetraohydrolase; a G4-amylase; a Glucan 1,4-alpha-maltohydrolase; a maltogenic alpha-amylase; a cyclodextrin glucanotransferase; a CGTase; a glucoamylase; an Endo-1,4-beta-xylanase; a xylanase; a cellulase; an Oxidoreductases; a Phospholipase A1; a Phospholipase A2; a Phospholipase C; a Phospholipase D; a Galactolipase, triacylglycerol lipase, an arabinofuranosidase, a transglutaminase, a pectinase, a pectate lyase, a protease, or any combination thereof.

9. The method of embodiment 1, wherein the lipase is active at a range from pH 4.0 to pH 12.0.

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10. The method of embodiment 1, wherein the lipase is active at a temperature range from 20 ° C to 60° C.

11. A method for increasing the volume of a baked product without the addition of diacetyl tartaric acid esters of monoglycerides (DATEM) comprising:

(a) providing a dough;

(b) providing a lipase, wherein the lipase has lipase activity and comprises an amino acid sequence with at least 90% sequence identity to: SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11; and

(c) combining the lipase of (b) with the dough of (a) without the addition of DATEM and baking the combination to generate the baked product having an increased volume.

12. The method of embodiment 11, wherein the dough is a composition comprising: a flour, a salt, water, and yeast.

13. The method of embodiment 12, wherein the flour is selected from the group consisting of: almond flour, coconut flour, chia flour, corn flour, barley flour, spelt flour, soya flour, hemp flour, potato flour, quinoa, teff flour, rye flour, amaranth flour, arrowroot flour, chick pea (garbanzo) flour, cashew flour, flax meal, macadamia flour, millet flour, sorghum flour, rice flour, tapioca flour, and any combination thereof.

14. The method of embodiment 12, wherein the yeast is selected from the group consisting of: baker's yeast, cream yeast, compressed yeast, cake yeast, active dry yeast, instant yeast, osmotolerant yeasts, rapid-rise yeast, deactivated yeast, nutritional yeast, brewer's yeast, distiller's and wine yeast.

15. The method of embodiment 11, wherein the lipase is a polypeptide having an amino acid sequence selected from the group consisting of: SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11.

16. The method of embodiment 11, wherein the lipase has lipase activity and is at least 97% identical to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11.

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17. The method of embodiment 11, wherein the lipase is a polypeptide encoded by a nucleic acid sequence that encodes that amino acid sequence selected from the group consisting of: SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11.

18. The method of embodiment 11, further comprising the addition of a second enzyme.

19. The method of embodiment 11, wherein the second enzyme comprises a second lipase, an Alpha-amylase; a Glucan 1, 4-alpha-maltotetraohydrolase; an exo-maltotetraohydrolase; a G4-amylase; a Glucan 1,4-alpha-maltohydrolase; a maltogenic alpha-amylase; a cyclodextrin glucanotransferase; a CGTase; a glucoamylase; an Endo-1,4-beta-xylanase; a xylanase; a cellulase; an Oxidoreductases; a Phospholipase A1; a Phospholipase A2; a Phospholipase C; a Phospholipase D; a Galactolipase, triacylglycerol lipase, an arabinofuranosidase, a transglutaminase, a pectinase, a pectate lyase, a protease, or any combination thereof.

20. The method of embodiment 11, wherein the lipase is active at a range from pH 4.0 to pH 12.0.

21. The method of embodiment 11, wherein the lipase is active at a temperature range from 20 ° C to 60° C.

Example 1: Lipase Expression and Purification

Expression

The enzymes were obtained by constructing expression plasmids containing the encoding polynucleotide sequences, transforming into *Pichia pastoris (Komagataella phaffii)* and growing the resulting expression strains in the following way. Fresh Pichia Pastoris cells of the expression strains were obtained by spreading the glycerol stocks of sequence-confirmed strains onto Yeast extract Peptone Dextrose (YPD) agar plates containing Zeocin. After 2 days, starter seed cultures of the production strains were inoculated into 100 mL of Buffered Glycerol complex Medium (BMGY) medium using cells from these plates, and grown for 20-24 hours at 30°C and 225-250 rpm. Seed cultures were scaled up by transferring suitable amounts into 2-4 L of BMMY medium in a baffled Fermentor. Fermentations were carried out at 30°C and under 1100 rpm of agitation, supplied via flat-blade impellers, for 48-72 hours. After the initial batch-phase of fermentation, sterile-

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filtered Methanol was added as feed whenever the dissolved oxygen level in the culture dipped below 30%. Alternatively, feed was added every 3 hours at 0.5% v/v of the starting batch culture. The final fermentation broth was centrifuged at 7000xg for 30 mins at 4°C to obtain the cell-free supernatant.

Purification

After filtering through cheese-cloth, the cell-free supernatants were ultrafiltered using a lab-scale tangential flow filtration (TFF) system with a molecular weight cut-off of 5 kD (SpectrumLabs). Samples were first concentrated 10-20X and then buffer-exchanged 5X into 50 mM HEPES pH 7.5. The resultant retentate was centrifuged at 27000xg for 1 hour, and then sterile filtered through 0.2 µm filters to remove any production organisms or particulate matter. Total protein content of the final samples was determined using the Braford assay. Lipases were either kept in solution at -20°C or lyophilized to form powder. In some cases, lipase solutions were sprayed on whole grain flour at 0.5 mg lipase per g flour, followed by drying at 40°C.

Lipase	MW, kDa	pI	Origin
Lip24	50.73	4.38	*Pseudomonas sp*
Lip49	35.6	4.58	*Moritella marina*
Lip61	34.12	7.06	*Fusarium solani*
Lip62	34.13	7.06	*Fusarium solani* Jallouli et. al. "The galactolipase activity of Fusarium solani (phospho)lipase." Biochim Biophys Acta. 2015 Mar;1851(3):282-9. doi: 10.1016/j.bbalip.2014.12.010. Epub 2014 Dec 18. PMID: 25529980
Lip70	34.05	4.37	*Colletotrichum fiorinae*
Lip88	34.19	7.12	*Fusarium solani* US6645749-SEQ ID NO:2
PANAMORE GOLDEN 2.2 (DSM)	36.9	5.19	*Fusarium culmorum* WO2009106575-SEQ ID NO:2
LIPOPAN F (Novozymes)	36.56	6.85	*Fusarium oxysporum* WO1998026057-SEQ ID NO:2

Example 2: Lipase Activity

Artificial substrate

Lipase activity was determined using the artificial substrate p-nitrophenyl octanoate (C8-PNP, Sigma 21742), by detecting spectrophotometrically the chromogenic product p-nitrophenyl (PNP). C8-PNP was dissolved at 8 mM in 2- ethoxyethanol (Alfa Aesar), then diluted to 0.4 mM into 50 mM Hepes pH 7.5, 0.1 M NaCl (substrate assay buffer). Lipase stock was added to the substrate assay buffer at final concentrations between 0.1-1 µg/mL, then PNP formation was

monitored immediately at 30°C for 15 minutes by absorbance at 405 nm in a plate reader. The linear slope of A405 versus time and a standard PNP curve were used to calculate the enzyme activity per µg of enzyme. Similarly, the same assay was used to measure the activity: a) at different pH values (4.0-12.0), using the appropriate pH buffers and the PNP standard curve at that pH value; b) at different temperatures (25°C-65°C); c) in presence of different cofactors or salt concentrations (Ca2+, Mg2+, Zn2+, Na+, Cl-, EDTA); d) with different PNP substrates of various fatty acid chain length (C4-C18, Sigma). The results are shown in the table below.

Lipase	Optimum			
	pH	Temperature °C	Cofactor	Fatty Acid Chain Length
Lip24	7.5-8.0	30-35	Ca2+	C8~C14~C16>C4>C18
Lip49	8.5-10.5	35-40	Mg2+	C8>C14>C16~C18>C4
Lip61	8.5-10.0	30-50	None	C8~C14>C16>C4~C18
Lip62	8.5-10.5	25-40	None	C8>C14>C16>C4~C18
Lip70	7.0	25	None	C14>C16~C8>C4~C18
Lip88	8.0-10.5	37-55	None	C8>C14>C16>C4~C18
PANAMORE GOLDEN 2.2	8.5-10.0	30.0	None	C10

Natural lipid substrates

Alternatively, lipase activity was determined using natural lipid substrates and fluorogenic pH indicators detecting the pH change due to free fatty acid accumulation during hydrolysis. Natural substrates were isolated from flour as described below (MGDG= monogalactosyl diglyceride, DGDG= digalactosylactosyl diglyceride and TAGs= triacylglycerols) or PC from soy lecithin (PC= phosphatidyl choline). Natural substrate stock solutions were prepared at 5 mM final concentration in 0.25% Na-deoxycholate, using sonication (1-5 minutes) to disperse the lipids homogenously. To measure activity at pH 7.0-7.5, lipases were diluted at 0.1-1 µg/mL into 2 mM substrate, 0.1% Na-deoxycholate, 125 ng/mL fluorescein, 5 mM CaCl2, 0.5 mM Hepes pH 7.5, followed by measuring fluorescence emission at 520 nm after excitation at 488 nm, at 30 °C for 15 minutes. The negative of linear slope of fluorescence versus time was used to calculate the lipase activity per µg enzyme. To measure activity at pH 7.5-8.0, lipases were diluted at 0.1-1 µg/mL into 2 mM substrate, 0.1% Na-deoxycholate, 250 ng/mL SNARF-1 (ThermoScientific S22801), 5 mM CaCl2, 1 mM Tris pH 8.0, followed by recording fluorescence emission at 580 nm after excitation at 514 nm, at 30 °C for 15 min. The linear slope of fluorescence versus time was used to calculate the lipase activity per µg enzyme.

Extraction of natural substrates from flour or soy lecithin

Flour type 550 (Vogtmühlen Illertissen) (1000 g) was added to a 6 L 4-necked round-bottom flask along with 2500 mL of methanol. The contents of the flask were then stirred for 1.5 hours using a mechanical stirring blade at room temperature. After this period, the mixture was allowed to settle and the solvent was decanted and filtered through a silica gel/Celite pad by vacuum filtration. The remaining wheat flour was then re-extracted with a further 2500 mL methanol as before.

After extraction, the entire content of the flask was filtered through silica/Celite as before and washed thoroughly with methanol to minimize the loss of lipid products. Both extracts were combined and concentrated using the rotary evaporator to give a golden-brown syrup. The combined extract was then purified through a silica pad packed into a sintered glass funnel in order to separate the fatty, non-polar components from the polar components i.e. MGDG and DGDG. The silica gel pad was prepared by filling a 500 mL sintered glass funnel with silica and applying a vacuum to ensure complete packing of the pad. The raw material was then carefully added to the silica pad using a Pasteur pipette to ensure even distribution of the sample. The sample was the eluted with n-heptane:acetone (1:1, 2 L), n-heptane:acetone (1:4, 2 L), acetone (1 L) and acetone-methanol (4:1,1 L). Fractions (1 L) were collected and, from TLC analysis, fraction 2 contained the bulk of the non-polar components (tri-, di-, monoglycerides), whereas, fractions 3-4 were observed to contain MGDG and fractions 5-6 contained DGDG. These fractions were separately concentrated using the rotary evaporator and further purified. The residue of fraction 2, (containing tri-, di-, monoglycerides) was purified performing a flash chromatography.

The column chromatography was run firstly using n-heptane followed by n-hepane:acetone (4:1) and n-hepane:acetone (1:1). The progress of the column chromatography was monitored via TLC analysis and the polarity of solvent system used for elution was increased accordingly. The fractions recovered from the column were then subjected to TLC analysis in order to evaluate which fractions could be combined in order to yield pure samples of tri-, di-, monoglycerides. The combined fractions were concentrated using the rotary evaporator. The residue of fractions 3-4, (MGDG containing fractions) was purified performing a flash chromatography. The column chromatography was run firstly using n-heptane followed by n-hepane:acetone (1:1). The progress of the column chromatography was monitored via TLC analysis and the polarity of solvent system used for elution was increased accordingly. The fractions recovered from the column were then subjected to TLC analysis in order to evaluate which fractions could be combined before concentration. The residue of fractions 5-6, (DGDG containing fractions) was purified performing a flash chromatography. The column

chromatography was run using n-hepane:acetone (1:1), n-heptane:acetone (1:4) and finally using only acetone. The progress of the column chromatography was monitored via TLC analysis and the solvent system was change accordingly. The fractions recovered from the column were then subjected to TLC analysis in order to evaluate which fractions could be combined before concentration.

Phospholipids were purified to remove triglycerides and free fatty acids from soy lecithin by acetone extraction. Soy lecithin (10 g) was mixed with acetone (30 ml) in a 50 ml tube and mixed for 10 minutes. The resulting slurry was centrifuged at 4000xg for 5 minutes and the acetone phase was removed and discarded. The insoluble phospholipids were extracted 3 further times with fresh acetone.

Abbreviation	Lipase Natural Substrates and Products
TAG	Triacyl glycerol
MGDG	Monogalactosyl diglyceride
DGDG	Digalactosyl diglyceride
NAPE	N-acylphosphatidyl ethanolamine
PC	Phosphatidyl choline
MAG	Monoacyl glycerol
DAG	Diacyl glycerol
FFA	Free fatty acid
MGMG	Monogalactosyl monoglyceride
DGMG	Digalactosyl monoglyceride

Lipase Name	Amino Acid SEQ ID No.	Nucleic Acid SEQ ID No.	Activity
LIP24	1	2	Triacylglycerol lipase
LIP49	3	4	Triacylglycerol lipase
LIP61	5	6	Galactolipase > Phospholipase > Triacylglycerol lipase
LIP62	7	8	Galactolipase > Phospholipase > Triacylglycerol lipase
LIP70	9	10	Phospholipase > Galactolipase
LIP88	11	12	Galactolipase > Phospholipase > Triacyglycerol lipase
PANAMORE GOLDEN 2.2	N/A	N/A	Triacylglycerol lipase> Galactolipase > Phospholipase

Lipolytic activity in dough assessed by HPLC

Simplified doughs were used to test the activity of lipases on several substrates at once and under desired conditions. Dough was prepared from 10 g flour (US King Arthur flour for bread), 200 mg salt and 5.9 ml water and enzymes were supplemented at either 4 or 40 µg

enzyme per dough. Doughs were mixed for 10 minutes by magnetic mixing then incubated in a humidity controlled chamber at 30°C for a total of 60 minutes. Samples for analysis were taken from each dough at 10 and 60 minutes. For lipid analysis, 2 g wet dough sample was added to a vial containing 2 ml 0.1 N HCl and 10 ml 1-butanol. The dough was dispersed in the solvents to extract the lipids by shear homogenization (VWR 250 Homogenizer, 20x200mm probe) for 30 seconds. The undissolved solids were then separated by centrifugation at 4000xg for 5 minutes at room temperature. The organic phase was removed and evaporated by centrifugal evaporation (Savant SpeedVac SC210A & Trap RVT5105), and the resulting solid was re-dissolved in isooctane:acetone:isopropanol (2:1:1) at 1/10 the original volume for analysis. Lipids were separated by HPLC (Agilent 1100 series) with a silica gel column (Chromolith Performance Si 100-4.6mm, Merck) and analyzed by ELSD (Agilent 1260 Infinity).

The chromatographic method for lipid separation was derived from Gerits, et. al. "Single run HPLC separation coupled to evaporative light scattering detection unravels wheat flour endogenous lipid redistribution during bread dough making" LWT-Food Science and Technology, 53 (2013) 426-433. Four samples, i.e. two time points and two enzyme doses, of each enzyme were used to determine if individual lipid classes increased, decreased or showed no change as a result of the enzyme treatment. Several of the enzymes tested show activity on a broad range of lipid classes as shown in the tables below and Figure 1.

Legend	
-	Consumption of compound
+	Production of compound
0	No change in compound

Lipase	TAG	MAG	FFA	MGDG	MGMG	DGDG	DGMG	NAPE
Lip24	-	+	+	0	0	0	0	0
Lip49	-	+	+	0	0	0	0	0
Lip61	-	-	+	-	+	-	0	-
Lip62	-	+	+	-	+	-	+	-
Lip70	0	-	+	-	+	-	+	-
Lip88	-	+	+	-	+	-	+	-
PANAMORE GOLDEN 2.2	-	-	+	-	+	-	+	-

Example 4: Baking Trials Pistolet Test

The baking performance of PANAMORE GOLDEN2.2, LIPOPAN F, LIP62, LIP61, LIP24, LIP49 dry lipase enzymes , and DATEM (LAMETOP 552) and also of PANAMORE GOLDEN2.2, LIPOPAN F, LIP62, LIP61, LIP24, LIP49, LIP88 lipase enzymes in solution , and DATEM (LAMETOP 552) were

tested in a fast straight dough system, the Pistolet test. Flour type 550 (Vogtmühlen Illertissen) (2000 g), 120 g compressed yeast, 40 g salt, 30 g glucose, 22 g wheat starch, 120 ppm ascorbic acid, 5 ppm Nutrilife® AM 100 (fungal alpha-amylase), 200 ppm Nutrilife® CS 30 (fungal xylanase, cellulase, fungal alpha-amylase) and 1180 g water was mixed in a Kemper SP 15 spiral mixer for 5.5 minutes at speed 1 and 0.5 minutes at speed 2, to a final dough temperature of 28°C. After a resting of 12 minutes, the dough was scaled to a 1500 g piece, rounded and proofed for another 12 minutes. Afterwards the dough was divided and rounded into 30 pieces of 50 g each by using an automatic dough divider and rounder. Then the dough pieces were proofed for 35 (normal proof) and 45 (extended proof) minutes at 35°C at relative humidity of 85%. After 12 minutes proofing time, a notch was pressed into the middle of the dough pieces. The proofed dough pieces were baked in a deck oven for 12 minutes at 240°C with 15 seconds steam addition.

The effects on the dough properties and on the final baked goods, were compared to a negative control and to a reference containing 0.4% (based on flour weight) DATEM (Lametop® 552). PANAMORE GOLDEN2.2 was dosed at 14 ppm and LIPOPAN F was dosed at 40 ppm.

The volume effect was determined by measurement of the length, width, and height of 15 rolls in relation to the weight. The negative control is defined as 0%. Dough properties were evaluated manually by a master baker and described in comparison to the negative control.

The results of the dry lipases and lipases in solution are shown in the tables below.

Dosage (µg lipase/ g flour)	**% Increase in Loaf Specific Volume Normal proof and dry Lipases (Pistolet)**						
	Lip62	Lip61	Lip24	Lip49	LIPOPAN F	PANAMORE GOLDEN 2.2	DATEM LT552
0.17			6				
0.33	6	5	9	1			
0.67	10	12	8	3	6	9	
1.34	11	10	5	3			
2.67				6			
5.34							
0.40%				3			11

Dosage (µg lipase/ g flour)	**% Increase in Loaf Specific Volume Extended proof and dry Lipases (Pistolet)**						
	Lip62	Lip61	Lip24	Lip49	LIPOPAN F	PANAMORE GOLDEN 2.2	DATEM LT552
0.167			13				
0.334	10	7	8	3			
0.668	14	13	10	5	10	12	
1.336	13	10	6	6			

Dosage (µg lipase/ g flour)	% Increase in Loaf Specific Volume Extended proof and dry Lipases (Pistolet)						
	Lip62	Lip61	Lip24	Lip49	LIPOPAN F	PANAMORE GOLDEN 2.2	DATEM LT552
2.672				6			
5.344							
0.40%				1			14

Dosage (µg lipase/ g flour)	% Increase in Loaf Specific Volume Normal proof and Lipases as solution (Pistolet)							
	Lip62	Lip61	Lip88	Lip49	Lip70	LIPOPAN F	PANAMORE GOLDEN 2.2	DATEM LT552
0.17	4							
0.33	9	4		3				
0.67	14	12	4	3		6	13	
1.34	13	11	6	6				
2.67	10	7	7	6	0			
3.33			9					
5.34		7		6	5			
0.40%								14

Dosage (µg lipase/ g flour)	% Increase in Loaf Specific Volume Extended proof and Lipases as solution (Pistolet)							
	Lip62	Lip61	Lip88	Lip49	Lip70	LIPOPAN F	PANAMORE GOLDEN 2.2	DATEM LT552
0.17	3							
0.33	7	6		8				
0.67	13	9	3	10		10	15	
1.34	12	12	7	10				
2.67	13	8	12	14	4			
3.33			13					
5.34		6		11	12			
0.40%								20

Dosage of Lip62 (µg/g flour)	DATEM (% of flour)	% Increase of Loaf Specific Volume (Pistolet)	
		Normal Proof	Extended proof
0	0	0	0
0	0.4	15	18
0.67	0	13	16
0.67	0.012	14	16
0.67	0.024	14	14
0.67	0.05	19	19

Dosage of Lip62 (μg/g flour)	DATEM (% of flour)	% of Loaf Specific Volume Increase (Pistolet)	
		Normal Proof	Extended proof
0	0	0	0
0	0.4	16	17
0.088	0.4	17	13
0.167	0.4	16	14
0.67	0.4	20	15

Example 5: Baking Trials – Baguette

The baking performance of PANAMORE GOLDEN 2.2, LIPOPAN F, LIP62 enzymes, and DATEM (Lametop 552) were tested in French baguette. Prior to the baking trials, each enzyme was tested for activity, which can vary between different enzymes, then each enzyme was tested to determine the optimum dosage for that enzyme, and finally the enzymes were added at the optimum dosage. Flour (see flour type below) (1000 g), 25 g compressed yeast, 20 g salt, 60 ppm ascorbic acid, 3 ppm Nutrilife® AM 100 (fungal alpha-amylase), 150 ppm Nutrilife® CS 30 (fungal xylanase, cellulase, fungal alpha-amylase) and 650 g water was mixed in a Kemper SP 15 spiral mixer for 8 minutes at speed 1 and 4 minutes at speed 2, to a final dough temperature of 27°C. After a resting of 35 minutes, the dough was divided into 350 g pieces, rounded and proofed for 15 minutes. Afterwards the dough pieces were molded and proofed for 120 (normal proof) and 150 (extended proof) minutes at 27°C at relative humidity of 75%. The proofed dough pieces were incised and baked in a deck oven for 25 minutes at 255°C, with steam addition after 30 seconds.

The effects on the dough properties and on the final baked goods, were compared to a negative control and to a reference containing 0.4% (based on flour) DATEM (Lametop® 552). Other controls were PANAMORE GOLDEN 2.2 (14 ppm) and LIPOPAN F (40 ppm). LIP62 was dosed at 60 ppm or 1.26 μg lipase/g flour.

The volume effect was determined by measuring the bread loavesvia a laser scanner (Micro Stable Systems Volscan). The negative control is defined as 0%. Dough properties were evaluated manually by a master baker and described in comparison to the negative control.

The results for the baguette using German flour (type 550 Vogtmühlen Illertissen) and Turkish flour baking trials are shown in the tables below.

Baguette Baking Trials	% of Loaf Specific Volume Increase on German flour	
	Normal Proof	Extended proof
PANOMORE GOLDEN 2.2	15	20
LIPOPAN F	11	19
Lip62	17	20
DATEM (LT552)	16	21

Baguette Baking Trials	% of Loaf Specific Volume Increase on Turkish flour	
	Normal Proof	Extended proof
PANOMORE GOLDEN 2.2	10	15
LIPOPAN F	19	19
Lip62	5	7
DATEM (LT552)	19	20

Example 6: Baking Trials – Sweet yeast dough

The baking performance of PANAMORE GOLDEN 2.2, LIPOPAN F, LIP62 enzymes, and DATEM (Lametop 552) were tested in sweet yeast dough. Prior to the baking trials, each enzyme was tested for activity, which can vary between different enzymes, then each enzyme was tested to determine the optimum dosage for that enzyme, and finally the enzymes were added at the optimum dosage. Flour type 550 (Vogtmühlen Illertissen) (2000 g), 140 g compressed yeast, 30 g salt, 200 g sugar, 200 g margarine, 100 g eggs, 50 ppm ascorbic acid, 200 ppm Nutrilife® CS 30 (fungal xylanase, cellulase, fungal alpha-amylase) and 900 g water was mixed in a Kemper SP 15 spiral mixer for 6.5 minutes at speed 1 and 1.5 minutes at speed 2, to a final dough temperature of 26°C. After a resting of 25 minutes, the dough was scaled to a 1500 g piece, rounded and proofed for another 20 minutes. Afterwards the dough was divided and rounded into 30 pieces of 50 g each by using an automatic dough divider and rounder. Then 8 dough pieces were given into a baking tin and proofed for 50 minutes at 35°C at relative humidity of 85%. The proofed dough pieces were baked in a deck oven for 35 minutes at 210°C/ 255°C under and upper heat, with 15 seconds steam addition.

The effects on the dough properties and on the final baked goods, were compared to a negative control and to a reference containing 0.4% (based on flour weight) DATEM (Lametop® 552). Other controls were PANAMORE GOLDEN 2.2 (4 ppm) or LIPOPAN F (25 ppm). LIP62 was dosed at 25 ppm (0.52 µg lipase/g flour).

The volume effect was determined by measuring the bread loavesvia a laser scanner (Micro Stable Systems Volscan). The negative control is defined as 0%. Dough properties were evaluated manually by a master baker and described in comparison to the negative control.

The results of the sweet dough and sponge & dough baking trails are shown in the table below.

Application Type	% Increase in Loaf Specific Volume on German Flour			
	PANAMORE GOLDEN 2.2	LIPOPAN F	Lip62	DATEM (LT552)
Sweet Yeast Dough	11	13	13	20

Example 7: Baking Trials – Sponge & Dough

The baking performance of PANAMORE GOLDEN 2.2, LIPOPAN F, LIP62 enzymes, and DATEM (Lametop 552) were tested in Sponge & Dough method. Prior to the baking trials, each enzyme was tested for activity, which can vary between different enzymes, then each enzyme was tested to determine the optimum dosage for that enzyme, and finally the enzymes were added at the optimum dosage. Flour type 550 (Vogtmühlen Illertissen) (1000 g), 5 g compressed yeast and 1000 g water was mixes and stored for 16 hours at either 4°C or room temperature. Afterwards 1000 g of flour type 550 (Vogtmühlen Illertissen), 55 g compressed yeast, 40 g salt, 40 g sugar, 40 g margarine, 60 ppm ascorbic acid, 150 ppm Nutrilife® CS 30 (fungal xylanase, cellulase, fungal alpha-amylase) and 160 g water was mixed in a Kemper SP 15 spiral mixer for 5.5 minutes at speed 1 and 0.5 minutes at speed 2, to a final dough temperature of 27°C. After a resting of 15 minutes, the dough was divided into 450 g pieces, rounded and proofed for 10 minutes. Afterwards the dough pieces were molded, given into a baking tin and proofed for 80 minutes at 35°C at relative humidity of 85%. The proofed dough pieces were baked in a deck oven for 30 minutes at 240°C/ 250°C under and upper heat, with 15 seconds steam addition.

The effects on the dough properties and on the final baked goods, were compared to a negative control and to a reference containing 0.4% (based on flour weight) DATEM (Lametop® 552). Other controls were PANAMORE GOLDEN2.2 (7 ppm) or LIPOPAN F (50 ppm). LIP62 was dosed at 1.2 µg lipase/g flour.

The volume effect was determined by measuring the bread loaves via a laser scanner (Micro Stable Systems Volscan). The negative control is defined as 100%. Dough properties were evaluated manually by a master baker and described in comparison to the negative control.

Sponge & Dough Trial	% Increase in Loaf Specific Volume on German Flour	
	4°C	Room temperature
Panamore Golden 2.2	2	-1
Lipopan F	7	2
LIP62	4	1
DATEM (LT552)	12	9

Example 8: Baking Trials – Chorleywood Bread Process

The baking performance of PANAMORE GOLDEN 2.2, LIPOPAN F, LIP62 enzymes, and DATEM (Lametop 552) were tested in Chorleywood Bread Process. Prior to the baking trials, each enzyme was tested for activity, which can vary between different enzymes, then each enzyme was tested to determine the optimum dosage for that enzyme, and finally the enzymes were added at the optimum dosage. UK flour (Heygates Standard) (3000), 240 g compressed yeast, 45 g salt, 60 g improver (wheat flour, calcium sulfate, soy flour, ascorbic acid, bacterial xylanase, fungal alpha amylase) and 2010 g water was mixed in a pressure vacuum mixer (Pentagon K5) until an energy input of 58.3 kW/h was reached, to a final dough temperature of 30°C. The dough was divided, without resting time, into 450 g pieces, rounded and proofed for 2 minutes. Afterwards the dough pieces were molded, given into two baking tins and proofed for 55 minutes at 35°C at relative humidity of 85%. Prior to baking one of the baking tins was used for a drop test, where the baking tin was dropped from a defined height. Then, the proofed dough pieces were baked in a deck oven for 25 minutes at 255°C/240°C under and upper heat, with 15 seconds steam addition.

The effects on the dough properties and on the final baked goods, were compared to a negative control and to a reference containing 0.4% (based on flour weight) DATEM (Lametop® 552). Other controls were PANAMORE GOLDEN2.2 (18 ppm) or LIPOPAN F (30 ppm). LIP62 was dosed at 40 ppm or 0.8 µg lipase/g flour.

The volume effect was determined by measuring the bread loafs via a laser scanner (Micro Stable Systems Volscan). The negative control is defined as 100%. Dough properties were evaluated manually by a master baker and described in comparison to the negative control.

Chorleywood Bread Process	% of Loaf Specific Volume Increase on UK flour	
	Normal Proof	After Drop Test
Panamore Golden 2.2	15	3
Lipopan F	17.5	-7
LIP62	16	37
DATEM (LT552)	34	23.5

Claims

1. A method for increasing the volume of a baked product comprising:

(a) providing a dough;

(b) providing a lipase, wherein the lipase has lipase activity and comprises an amino acid sequence with at least 90% sequence identity to : SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11; and

(c) combining the lipase of (b) with the dough of (a) and baking the combination to generate the baked product having an increased volume.

2. The method of claim 1, wherein the dough is a composition comprising: a flour, a salt, water, and yeast.

3. The method of claim 2, wherein the flour is selected from the group consisting of: almond flour, coconut flour, chia flour, corn flour, barley flour, spelt flour, soya flour, hemp flour, potato flour, quinoa, teff flour, rye flour, amaranth flour, arrowroot flour, chick pea (garbanzo) flour, cashew flour, flax meal, macadamia flour, millet flour, sorghum flour, rice flour, tapioca flour, and any combination thereof.

4. The method of claim 2, wherein the yeast is selected from the group consisting of: baker's yeast, cream yeast, compressed yeast, cake yeast, active dry yeast, instant yeast, osmotolerant yeasts, rapid-rise yeast, deactivated yeast, nutritional yeast, brewer's yeast, distiller's and wine yeast.

5. The method of claim 1, wherein the lipase has lipase activity and is at least 97% identical to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11.

6. The method of claim 1, wherein the lipase is a polypeptide encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12.

7. The method of claim 1, further comprising the addition of a second enzyme.

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8. The method of claim 7, wherein the second enzyme comprises a second lipase, an Alpha-amylase; a Glucan 1, 4-alpha-maltotetraohydrolase; an exo-maltotetraohydrolase; a G4-amylase; a Glucan 1,4-alpha-maltohydrolase; a maltogenic alpha-amylase; a cyclodextrin glucanotransferase; a CGTase; a glucoamylase; an Endo-1,4-beta-xylanase; a xylanase; a cellulase; an Oxidoreductases; a Phospholipase A1; a Phospholipase A2; a Phospholipase C; a Phospholipase D; a Galactolipase, triacylglycerol lipase, an arabinofuranosidase, a transglutaminase, a pectinase, a pectate lyase, a protease, or any combination thereof.

9. The method of claim 1, wherein the lipase is active at a range from pH 4.0 to pH 12.0.

10. The method of claim 1, wherein the lipase is active at a temperature range from 20 ° C to 60° C.

11. A method for increasing the volume of a baked product without the addition of diacetyl tartaric acid esters of monoglycerides (DATEM) comprising:

(a) providing a dough;

(b) providing a lipase, wherein the lipase has lipase activity and comprises an amino acid sequence with at least 90% sequence identity to: SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11; and

(c) combining the lipase of (b) with the dough of (a) without the addition of DATEM and baking the combination to generate the baked product having an increased volume.

12. The method of claim 11, wherein the dough is a composition comprising: a flour, a salt, water, and yeast.

13. The method of claim 12, wherein the flour is selected from the group consisting of: almond flour, coconut flour, chia flour, corn flour, barley flour, spelt flour, soya flour, hemp flour, potato flour, quinoa, teff flour, rye flour, amaranth flour, arrowroot flour, chick pea (garbanzo) flour, cashew flour, flax meal, macadamia flour, millet flour, sorghum flour, rice flour, tapioca flour, and any combination thereof.

14. The method of claim 12, wherein the yeast is selected from the group consisting of: baker's yeast, cream yeast, compressed yeast, cake yeast, active dry yeast, instant yeast, osmotolerant

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yeasts, rapid-rise yeast, deactivated yeast, nutritional yeast, brewer's yeast, distiller's and wine yeast.

15. The method of claim 11, wherein the lipase is a polypeptide having an amino acid sequence selected from the group consisting of: SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11.

16. The method of claim 11, wherein the lipase has lipase activity and is at least 97% identical to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11.

17. The method of claim 11, wherein the lipase is a polypeptide encoded by a nucleic acid sequence that encodes that amino acid sequence selected from the group consisting of: SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11.

18. The method of claim 11, further comprising the addition of a second enzyme.

19. The method of claim 11, wherein the second enzyme comprises a second lipase, an Alpha-amylase; a Glucan 1, 4-alpha-maltotetraohydrolase; an exo-maltotetraohydrolase; a G4-amylase; a Glucan 1,4-alpha-maltohydrolase; a maltogenic alpha-amylase; a cyclodextrin glucanotransferase; a CGTase; a glucoamylase; an Endo-1,4-beta-xylanase; a xylanase; a cellulase; an Oxidoreductases; a Phospholipase A1; a Phospholipase A2; a Phospholipase C; a Phospholipase D; a Galactolipase, triacylglycerol lipase, an arabinofuranosidase, a transglutaminase, a pectinase, a pectate lyase, a protease, or any combination thereof.

20. The method of claim 11, wherein the lipase is active at a range from pH 4.0 to pH 12.0.

21. The method of claim 11, wherein the lipase is active at a temperature range from 20 ° C to 60° C.

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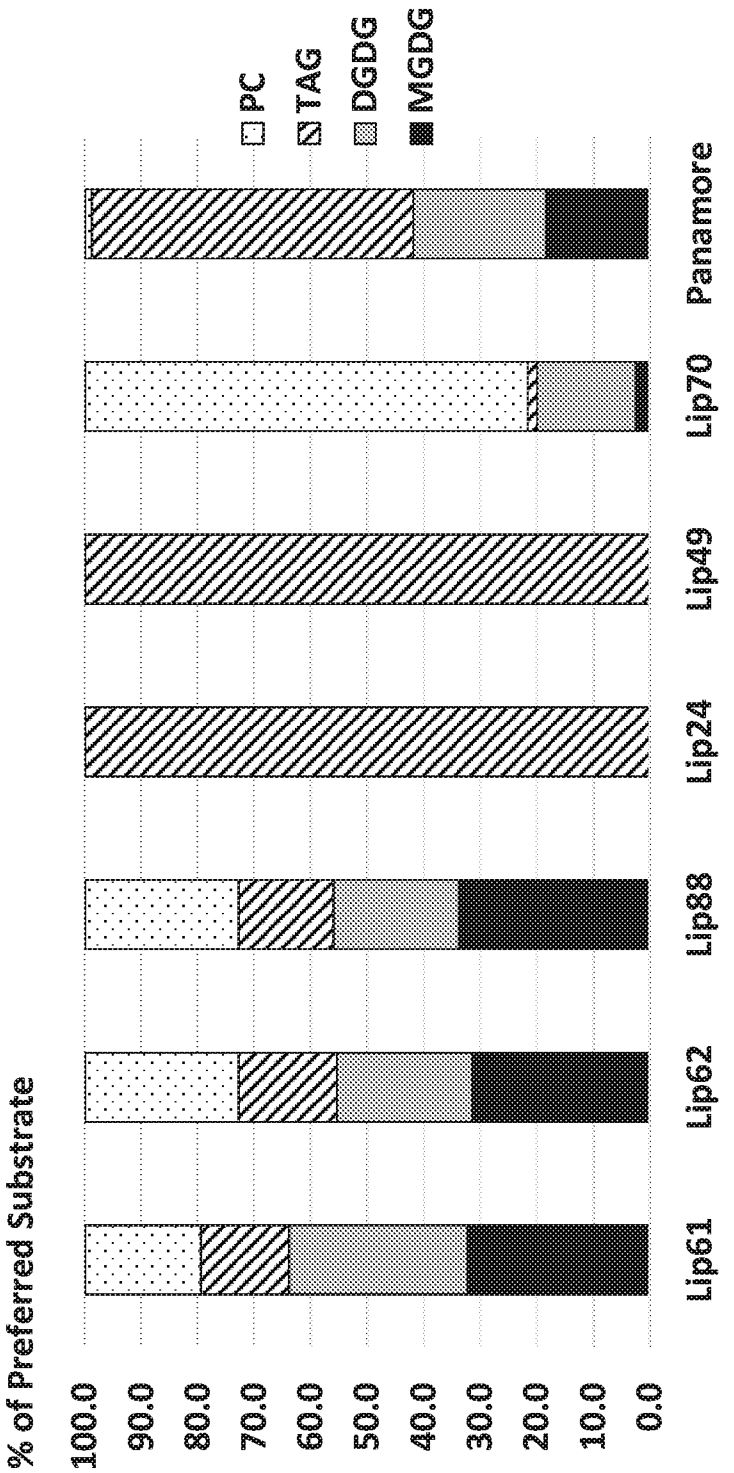
Figure 1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Lip62
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Lip49
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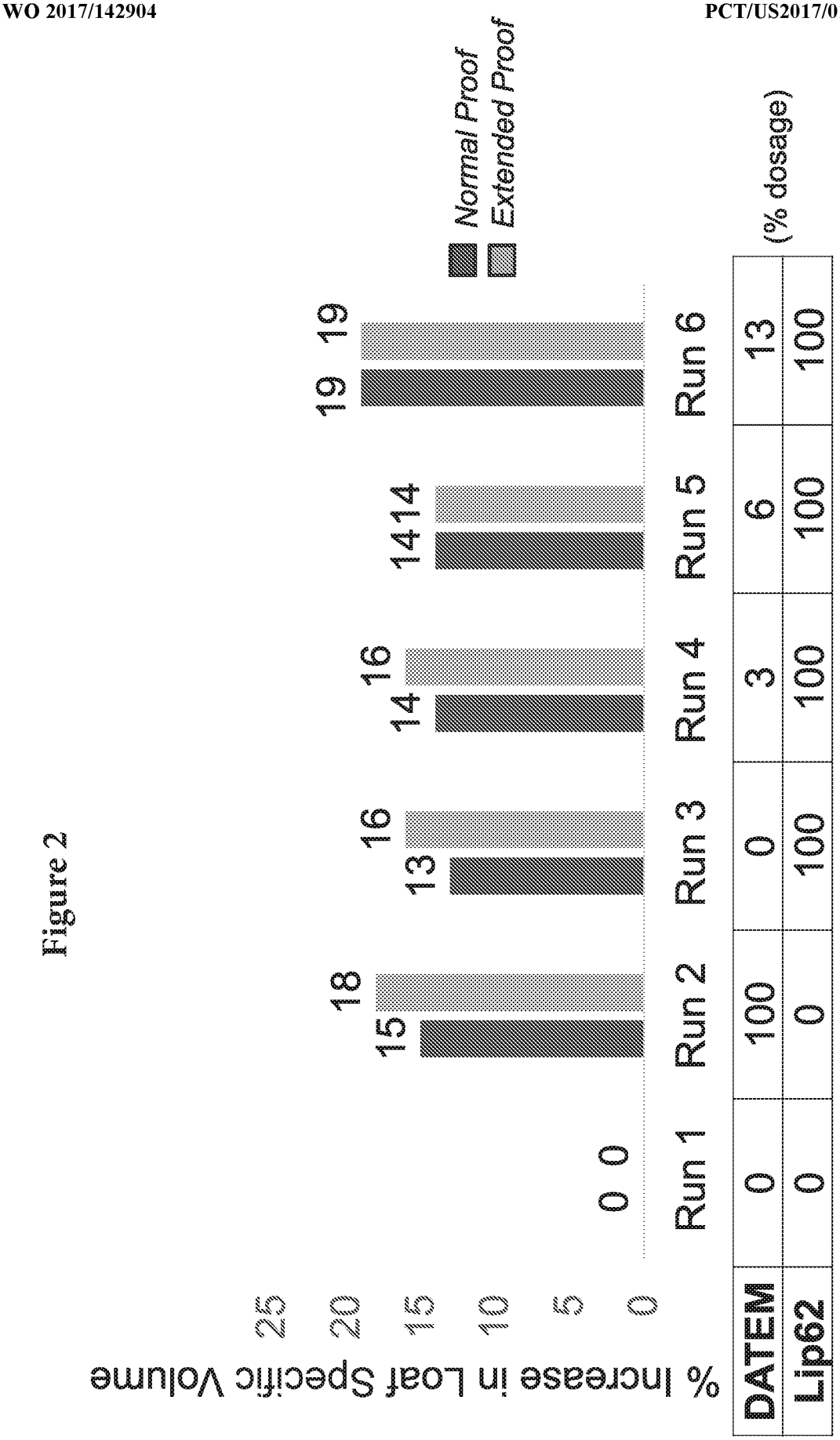
Panamore

Figure 2


% Increase in Loaf Specific Volume
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Normal Proof
Extended Proof
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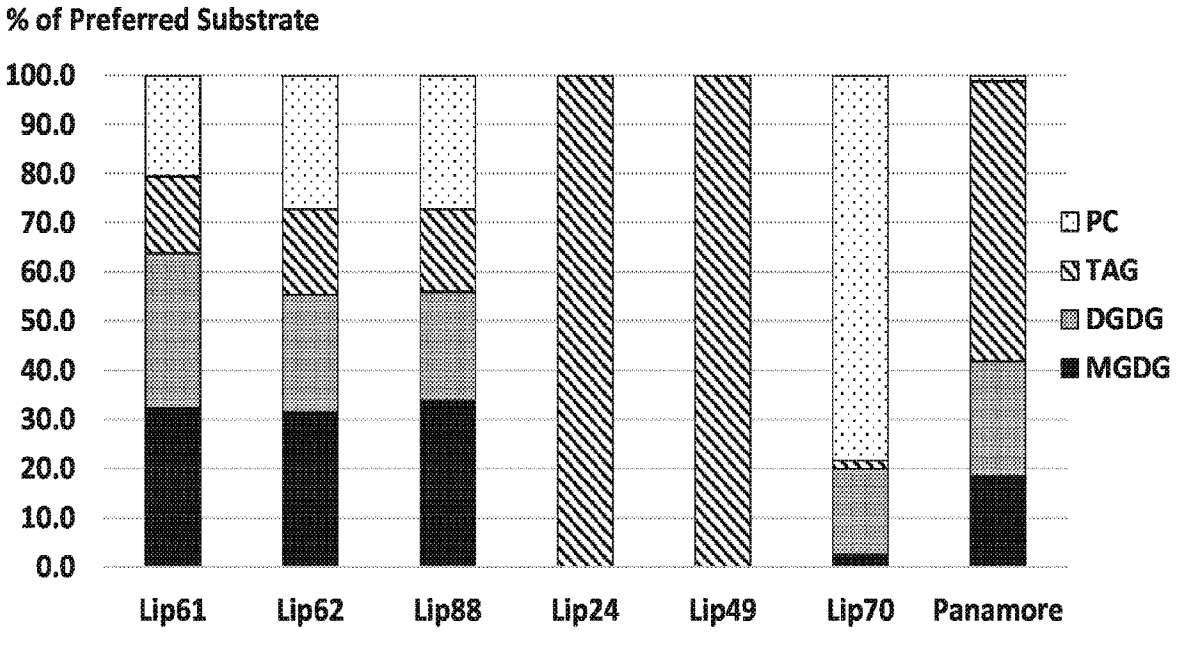
19 19
Run 1
Run 2
Run 3
Run 4
Run 5
Run 6
DATEM 0 100 0 3 6 13
Lip62 0 0 100 100 100 100
(% dosage)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DGDG
MGDG
Lip61
Lip62
Lip88
Lip24
Lip49
Lip70
Panamore