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(54) Title: USE OF GLUCOSYL TRANSFERASE TO PROVIDE IMPROVED TEXTURE IN FERMENTED MILK BASED PRODUCTS

(57) Abstract: The present teachings provide a method of making a yogurt product having increased thickness having the steps of providing milk; adding sucrose to the milk to form sweetened milk; contacting the sweetened milk with a glucosyl transferase to form an insoluble glucose polymer; inoculating with a starter culture; and fermenting to provide the yogurt product having increased thickness. Additional methods are provided.

USE OF GLUCOSYL TRANSFERASE TO PROVIDE IMPROVED TEXTURE IN FERMENTED MILK BASED PRODUCTS

BACKGROUND OF THE INVENTION

Texture is a key quality and value parameter of fresh fermented dairy products, such as yogurts and fermented milks. Yogurt texture as relates to consumer eating sensation heavily impacts consumer perception. Today, stabilizers such as starch are common additives to yogurt to enhance texture. Yogurts containing starch, however, require special handling during processing so as not to lose the texture created by the starch through shear forces. The use of starch also adds to the expense of the yogurt. In addition, it is well known that starch negatively impacts yogurt in several ways. First, starch diminishes the "shininess" of yogurt, negatively impacting consumer visual perception. Moreover, added starch often leads to an undesirable sensory dryness of the yogurt.

In addition to starch, protein and fat levels in yogurt also contribute in a significant way to texture. Moreover, fat levels also impact taste. Modifying the protein level or fat level is a way to work with the cost profile and the nutritional profile of the yogurt. When reducing the content of any of these it is common to use other ingredients to compensate for texture or taste loss, typically by adding ingredients such as starch.

There is a need for adding texture to fermented dairy products that does not include the addition of starch or other stabilizers.

SUMMARY OF THE INVENTION

In accordance with an aspect of the present invention, a method is presented of making a yogurt product having improved texture, improved texture being increased thickness and/or mouthfeel, having the steps of: providing milk; adding sucrose to the milk to form sweetened milk; contacting the sweetened milk with a glucosyl transferase to form an insoluble glucose polymer; inoculating with a starter culture; and fermenting to provide the yogurt product having improved texture which is increased thickness and/or increased mouthfeel.

Optionally, the milk is cow's milk. Optionally, the milk is selected from the group consisting of raw milk, pre-pasteurized milk, whole milk, skim milk, reconstituted milk, lactase treated milk, reduced lactose milk, lactose free milk and condensed milk. Optionally, the milk is raw milk.

Optionally, the method has the additional steps of homogenizing and pasteurizing the milk. Optionally, the step of contacting with glucosyl transferase is performed after the steps of homogenizing and pasteurizing. Optionally, the step of contacting with glucosyl transferase is performed before the steps of homogenizing and pasteurizing.

Optionally, the sucrose is added to constitute about 0.1 to 12% (w/w). Optionally, the sucrose is added to constitute about 2 to 8% (w/w). Optionally, the sucrose is added to constitute about 4 to 6% (w/w).

Optionally, the glucosyl transferase is an enzyme which has at least 70% sequence identity to an enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15). Optionally, the glucosyl transferase is an enzyme which has at least 80% sequence identity to an enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15). Optionally, the glucosyl transferase is an enzyme which has at least 90% sequence identity to an enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15). Optionally, the glucosyl transferase is an enzyme which has at least 95% sequence identity to an enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and

GTF3929 (SEQ ID NO: 15). Optionally, the glucosyl transferase is selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15). Optionally, the glucosyl transferase is GTFJ (SEQ ID NO: 1).

Optionally, the glucosyl transferase is present in the milk in an amount from about 0.005 mg per 100 ml milk to 15 mg per 100 ml milk. Optionally, the glucosyltransferase is present in an amount from about 0.03 mg per 100 ml milk to about 12.5 mg per 100 ml milk.

Optionally, the GTFJ is present in an amount from about 0.033 mg per 100 ml milk to about 12.5 mg per 100 ml milk. Optionally, the GTFJ is present in an amount from about 0.3 mg per 100 ml milk to about 5.0 mg per 100 ml milk.

Optionally, the glucosyl transferase is GTF300 (SEQ ID NO: 2). Optionally, the GTF300 is present in an amount from about 0.033 mg per 100 ml to about 12.5 mg per 100 ml milk. Optionally, the GTF300 is present in an amount from about 1.25 mg per 100 ml milk to about 5 mg per 100 ml milk.

Optionally, the increased texture is increased thickness. Optionally, the thickness is increased by 30% or more as compared with a control sample (no GTF enzyme). Optionally, the thickness is increased by 50% or more. Optionally, the thickness is increased by 70% or more. Optionally, the thickness is increased by 90% or more. Optionally, the thickness is increased by 100% or more. Optionally, the thickness is increased by 110% or more. Optionally, the thickness is increased by 120% or more.

Optionally, the increased texture is increased mouthfeel. Optionally, the mouthfeel is increased by 30% or more as compared with a control sample (no GTF enzyme). Optionally, the mouthfeel is increased by 50% or more. Optionally, the mouthfeel is increased by 70% or more. Optionally, the mouthfeel is increased by 90% or more. Optionally, the mouthfeel is increased by 100% or more. Optionally, the mouthfeel is increased by 110% or more. Optionally, the mouthfeel is increased by 120% or more.

Optionally, the method includes the further steps of cooling the yogurt of to a temperature of between 5 and 10° C to provide a chilled yogurt; and pouring the chilled yogurt into preformed containers. Optionally, the containers provide a single serving of yogurt.

Optionally, the milk is low fat milk to provide a low fat yogurt. Optionally, the milk is non-fat milk to provide a non-fat yogurt.

Optionally, the protein content of the milk is adjusted to at least about 3% (w/w). Optionally, the protein content of the milk is adjusted to at least about 3.5%. Optionally, the protein content of the milk is adjusted to at least about 3.7% (w/w). Optionally, the protein content of the milk is adjusted to at least about 3.8 % (w/w). Optionally, the protein content of the milk is adjusted to at least about 3.9 % (w/w). Optionally, the protein content of the milk is adjusted to at least about 4.0 % (w/w).

In accordance with an aspect of the present invention, a yogurt is presented which is made according to any or the preceding methods. Optionally, the yogurt contains pectin.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a flow diagram for inoculation of milk with culture and treatment with GTF enzyme.

FIG. 2 depicts the thickness and mouthfeel of GTF300 treated and control yogurt after 5 days using three different starter cultures: FIG. 2A (YO-MIX 860), FIG. 2B (YO-MIX 495) and FIG. 2C (YO-MIX 465).

FIG. 3 depicts the thickness and mouthfeel of enzyme treated and control yogurt after 28 days with GTF300 addition at the same time as culture inoculation using three different starter cultures: FIG. 3A (YO-MIX 860), FIG. 3B (YO-MIX 495) and FIG. 3C (YO-MIX 465).

FIG. 4 depicts a flow diagram for addition of enzyme prior to pasteurization and homogenization.

FIG. 5 depicts the thickness and mouthfeel of GTF300 treated and control yogurt after 7 days with enzyme addition before pasteurization and homogenization using four different starter cultures: FIG. 5A (YO-MIX 495), FIG. 5B (YO-MIX 465), FIG. 5C (YO-MIX 860) and FIG. 5D (YO-MIX 204).

FIG. 6 depicts the thickness and mouthfeel of enzyme treated and control yogurt after 28 days with GTF300 addition before pasteurization and homogenization using four different starter

cultures: FIG. 6A (YO-MIX 860), FIG. 6B (YO-MIX 495), FIG. 6C (YO-MIX 465) and FIG 6D (YO-MIX 204).

FIG. 7A (2% sucrose) and 7B (4% sucrose) thickness and mouthfeel as evaluated with GTF300 addition before pasteurization and homogenization.

FIG. 8 depicts the effect of yogurt cooling to different temperatures on texture and mouthfeel generated by GTF300 after filling.

FIG. 9 depicts the effect of GTFJ on thickness and mouthfeel. FIG. 9A shows effect of GTFJ as function of enzyme concentration and FIG. 9B shows the effect of 3.7% protein plus GTFJ as compared with a 4% protein yogurt without enzyme.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file named 20180703_NB41287_ST25.txt created on July 3, 2018 and having a size of 174 kilobytes and is filed concurrently with the specification. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

BRIEF DESCRIPTION OF THE SEQUENCE IDs.

SEQ ID NO: 1 is the amino acid sequence of GTFJ.

SEQ ID NO: 2 is the amino acid sequence of GTF300.

SEQ ID NO: 3 is the amino acid sequence of GTF0874.

SEQ ID NO: 4 is the amino acid sequence of GTF6855.

SEQ ID NO: 5 is the amino acid sequence of GTF2379.

SEQ ID NO: 6 is the amino acid sequence of GTF7527.

SEQ ID NO: 7 is the amino acid sequence of GTF1724.

SEQ ID NO: 8 is the amino acid sequence of GTF0544.

SEQ ID NO: 9 is the amino acid sequence of GTF5926.

SEQ ID NO: 10 is the amino acid sequence of GTF4297.

SEQ ID NO: 11 is the amino acid sequence of GTF5618.

SEQ ID NO: 12 is the amino acid sequence of GTF2765.

SEQ ID NO: 13 is the amino acid sequence of GTF2919.

SEQ ID NO: 14 is the amino acid sequence of GTF2678.

SEQ ID NO: 15 is the amino acid sequence of GTF3929.

DETAILED DISCLOSURE OF THE INVENTION

Detailed description of the inventions:

The practice of the present teachings will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, and biochemistry, which are within the skill of the art. Such techniques are explained fully in the literature, for example, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989); Oligonucleotide Synthesis (M. J. Gait, ed., 1984; Current Protocols in Molecular Biology (F. M. Ausubel et al., eds., 1994); PCR: The Polymerase Chain Reaction (Mullis et al., eds., 1994); Gene Transfer and Expression: A Laboratory Manual (Kriegler, 1990), and The Alcohol Textbook (Ingledew et al., eds., Fifth Edition, 2009), and Essentials of Carbohydrate Chemistry and Biochemistry (Lindhorste, 2007).

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present teachings belong. Singleton, et al., Dictionary of Microbiology and Molecular Biology, second ed., John Wiley and Sons, New York (1994), and Hale & Markham, The Harper Collins Dictionary of Biology, Harper Perennial, NY (1991) provide one of skill with a general dictionary of many of the terms used in this invention. Any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present teachings.

Numeric ranges provided herein are inclusive of the numbers defining the range.

Definitions:

As used herein, “alpha (1-3) glucan” refers to an oligo or polysaccharide containing alpha 1-3 bonds between glucose monomers.

The terms "glucosyl transferase", “glucosyl transferase enzyme”, "GTF enzyme", and "GTF” are used interchangeably herein. Glucosyl transferases catalyze the synthesis of high molecular weight D-glucose polymers named glucan from sucrose. GTF enzymes are classified under the glycoside hydrolase family 70 (GH70) according to the CAZy (Carbohydrate-Active EnZymes) database (Cantarel et al., Nucleic Acids Res. 37:D233-238, 2009).

The terms, "wild-type," "parental," or "reference," with respect to a polypeptide, refer to a naturally-occurring polypeptide that does not include a man-made substitution, insertion, or deletion at one or more amino acid positions. Similarly, the terms "wild-type," "parental," or "reference," with respect to a polynucleotide, refer to a naturally-occurring polynucleotide that does not include a man-made nucleotide change. However, note that a polynucleotide encoding a wild-type, parental, or reference polypeptide is not limited to a naturally-occurring polynucleotide, and encompasses any polynucleotide encoding the wild-type, parental, or reference polypeptide.

Reference to the wild-type polypeptide is understood to include the mature form of the polypeptide. A "mature" polypeptide or variant, thereof, is one in which a signal sequence is absent, for example, cleaved from an immature form of the polypeptide during or following expression of the polypeptide.

The term "variant," with respect to a polypeptide, refers to a polypeptide that differs from a specified wild-type, parental, or reference polypeptide in that it includes one or more naturally-occurring or man-made substitutions, insertions, or deletions of an amino acid. Similarly, the term "variant," with respect to a polynucleotide, refers to a polynucleotide that differs in nucleotide sequence from a specified wild-type, parental, or reference polynucleotide. The identity of the wild-type, parental, or reference polypeptide or polynucleotide will be apparent from context.

The term "recombinant," when used in reference to a subject cell, nucleic acid, protein or vector, indicates that the subject has been modified from its native state. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell, or express native genes at different levels or under different conditions than found in nature. Recombinant nucleic acids differ from a native sequence by one or more nucleotides and/or are operably linked to heterologous sequences, *e.g.*, a heterologous promoter in an expression vector. Recombinant proteins may differ from a native sequence by one or more amino acids and/or are fused with heterologous sequences. A vector comprising a nucleic acid encoding a glucosyl transferase is a recombinant vector.

The terms "recovered," "isolated," and "separated," refer to a compound, protein (polypeptides), cell, nucleic acid, amino acid, or other specified material or component that is removed from at least one other material or component with which it is naturally associated as

found in nature. An "isolated" polypeptides, thereof, includes, but is not limited to, a culture broth containing secreted polypeptide expressed in a heterologous host cell.

The term "polymer" refers to a series of monomer groups linked together. A polymer is composed of multiple units of a single monomer. As used herein the term "glucose polymer" refers to glucose units linked together as a polymer. As long as there are at least three glucose units, the glucose polymer may contain non-glucose sugars such as lactose or galactose.

The term "amino acid sequence" is synonymous with the terms "polypeptide," "protein," and "peptide," and are used interchangeably. Where such amino acid sequences exhibit activity, they may be referred to as an "enzyme." The conventional one-letter or three-letter codes for amino acid residues are used, with amino acid sequences being presented in the standard amino-to-carboxy terminal orientation (*i.e.*, N→C).

The term "nucleic acid" encompasses DNA, RNA, heteroduplexes, and synthetic molecules capable of encoding a polypeptide. Nucleic acids may be single stranded or double stranded, and may be chemical modifications. The terms "nucleic acid" and "polynucleotide" are used interchangeably. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and the present compositions and methods encompass nucleotide sequences that encode a particular amino acid sequence. Unless otherwise indicated, nucleic acid sequences are presented in 5′-to-3′ orientation.

The terms "transformed," "stably transformed," and "transgenic," used with reference to a cell means that the cell contains a non-native (*e.g.*, heterologous) nucleic acid sequence integrated into its genome or carried as an episome that is maintained through multiple generations.

The term "introduced" in the context of inserting a nucleic acid sequence into a cell, means "transfection", "transformation" or "transduction," as known in the art.

A "host strain" or "host cell" is an organism into which an expression vector, phage, virus, or other DNA construct, including a polynucleotide encoding a polypeptide of interest (*e.g.*, a glucosyl transferase) has been introduced. Exemplary host strains are microorganism cells (*e.g.*, bacteria, filamentous fungi, and yeast) capable of expressing the polypeptide of interest. The term "host cell" includes protoplasts created from cells.

The term "heterologous" with reference to a polynucleotide or protein refers to a polynucleotide or protein that does not naturally occur in a host cell.

The term "endogenous" with reference to a polynucleotide or protein refers to a polynucleotide or protein that occurs naturally in the host cell.

The term "expression" refers to the process by which a polypeptide is produced based on a nucleic acid sequence. The process includes both transcription and translation.

A "selective marker" or "selectable marker" refers to a gene capable of being expressed in a host to facilitate selection of host cells carrying the gene. Examples of selectable markers include but are not limited to antimicrobials (*e.g.*, hygromycin, bleomycin, or chloramphenicol) and/or genes that confer a metabolic advantage, such as a nutritional advantage on the host cell.

A "vector" refers to a polynucleotide sequence designed to introduce nucleic acids into one or more cell types. Vectors include cloning vectors, expression vectors, shuttle vectors, plasmids, phage particles, cassettes and the like.

An "expression vector" refers to a DNA construct comprising a DNA sequence encoding a polypeptide of interest, which coding sequence is operably linked to a suitable control sequence capable of effecting expression of the DNA in a suitable host. Such control sequences may include a promoter to effect transcription, an optional operator sequence to control transcription, a sequence encoding suitable ribosome binding sites on the mRNA, enhancers and sequences which control termination of transcription and translation.

The term "operably linked" means that specified components are in a relationship (including but not limited to juxtaposition) permitting them to function in an intended manner. For example, a regulatory sequence is operably linked to a coding sequence such that expression of the coding sequence is under control of the regulatory sequences.

A "signal sequence" is a sequence of amino acids attached to the N-terminal portion of a protein, which facilitates the secretion of the protein outside the cell. The mature form of an extracellular protein lacks the signal sequence, which is cleaved off during the secretion process.

"Biologically active" refers to a sequence having a specified biological activity, such an enzymatic activity.

The term "specific activity" refers to the number of moles of substrate that can be converted to product by an enzyme or enzyme preparation per unit time under specific conditions. Specific activity is generally expressed as units (U)/mg of protein.

As used herein, "percent sequence identity" means that a particular sequence has at least a certain percentage of amino acid residues identical to those in a specified reference sequence,

when aligned using the CLUSTAL W algorithm with default parameters. *See* Thompson *et al.* (1994) *Nucleic Acids Res.* 22:4673-4680. Default parameters for the CLUSTAL W algorithm are:

Gap opening penalty:	10.0
Gap extension penalty:	0.05
Protein weight matrix:	BLOSUM series
DNA weight matrix:	IUB
Delay divergent sequences %:	40
Gap separation distance:	8
DNA transitions weight:	0.50
List hydrophilic residues:	GPSNDQEKR
Use negative matrix:	OFF
Toggle Residue specific penalties:	ON
Toggle hydrophilic penalties:	ON
Toggle end gap separation penalty	OFF.

Deletions are counted as non-identical residues, compared to a reference sequence. Deletions occurring at either termini are included. For example, a variant with five amino acid deletions of the C-terminus of the mature 617 residue polypeptide would have a percent sequence identity of 99% (612 / 617 identical residues × 100, rounded to the nearest whole number) relative to the mature polypeptide. Such a variant would be encompassed by a variant having "at least 99% sequence identity" to a mature polypeptide.

"Fused" polypeptide sequences are connected, *i.e.*, operably linked, via a peptide bond between two subject polypeptide sequences.

The term "filamentous fungi" refers to all filamentous forms of the subdivision Eumycotina, particularly Pezizomycotina species.

The term "about" refers to ± 5% to the referenced value.

"Lactase treated milk" means milk treated with lactase to reduce the amount of lactose sugar.

"Reduced lactose milk" means milk wherein the percentage of lactose is about 2% or lower.

"Lactose free milk" means milk wherein the percentage of lactose is about 0.5% or lower.

The terms "GTFJ" means the glucosyl transferase enzyme having the sequence as set forth in SEQ ID NO:1.

The term "GTF300" means the glucosyl transferase having the sequence as set forth in SEQ ID NO: 2.

The term "texture" as used herein to refer to a yogurt or fermented milk products means the thickness of the yogurt and/or the sensory perception of mouthfeel or both. An "improvement" in texture means an increase in thickness and/or an increase in the sensory perception of mouthfeel or both. Unless otherwise noted, as used herein the "thickness" of a yogurt or fermented milk beverage means the apparent viscosity extracted at shear rate of 10 Hz. Thus, an increase in apparent viscosity at a shear rate of 10 Hz indicates an increase in thickness. The apparent viscosity extracted at shear rate 200 Hz is correlated to "mouthfeel". Hence, an increase in apparent viscosity at a shear rate of 200 Hz indicates an increase in mouthfeel.

Additional mutations

In some embodiments, the present glucosyl transferases further include one or more mutations that provide a further performance or stability benefit. Exemplary performance benefits include but are not limited to increased thermal stability, increased storage stability, increased solubility, an altered pH profile, increased specific activity, modified substrate specificity, modified substrate binding, modified pH-dependent activity, modified pH-dependent stability, increased oxidative stability, and increased expression. In some cases, the performance benefit is realized at a relatively low temperature. In some cases, the performance benefit is realized at relatively high temperature.

Furthermore, the present glucosyl transferases may include any number of conservative amino acid substitutions. Exemplary conservative amino acid substitutions are listed in the following Table.

Conservative amino acid substitutions

For Amino Acid	*Code*	*Replace with any of*
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln

Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, b-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4- carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

The reader will appreciate that some of the above mentioned conservative mutations can be produced by genetic manipulation, while others are produced by introducing synthetic amino acids into a polypeptide by genetic or other means.

The present glucosyl transferases may be "precursor," "immature," or "full-length," in which case they include a signal sequence, or "mature," in which case they lack a signal sequence. Mature forms of the polypeptides are generally the most useful. Unless otherwise noted, the amino acid residue numbering used herein refers to the mature forms of the respective glucosyl transferase polypeptides. The present glucosyl transferase polypeptides may also be truncated to remove the N or C-termini, so long as the resulting polypeptides retain glucosyl transferase activity.

The present glucosyl transferases may be a "chimeric" or "hybrid" polypeptide, in that it includes at least a portion of a first glucosyl transferase polypeptide, and at least a portion of a second glucosyl transferase polypeptide. The present glucosyl transferases may further include heterologous signal sequence, an epitope to allow tracking or purification, or the like. Exemplary heterologous signal sequences are from *B. licheniformis* amylase (LAT), *B. subtilis* (AmyE or AprE), and *Streptomyces* CelA.

Production of glucosyl transferases

The present glucosyl transferases can be produced in host cells, for example, by secretion or intracellular expression. A cultured cell material (*e.g.*, a whole-cell broth) comprising a glucosyl transferase can be obtained following secretion of the glucosyl transferase into the cell medium. Optionally, the glucosyl transferase can be isolated from the host cells, or even isolated from the cell broth, depending on the desired purity of the final glucosyl transferase. A gene encoding a glucosyl transferase can be cloned and expressed according to methods well known in the art. Suitable host cells include bacterial, fungal (including yeast and filamentous fungi), and plant cells (including algae). Particularly useful host cells include *Aspergillus niger*, *Aspergillus oryzae* or *Trichoderma reesei*. Other host cells include bacterial cells, *e.g.*, *Bacillus subtilis* or *B. licheniformis*, as well as *Streptomyces,* and *E. Coli*.

The host cell further may express a nucleic acid encoding a homologous or heterologous glucosyl transferase, *i.e.*, a glucosyl transferase that is not the same species as the host cell, or one or more other enzymes. The glucosyl transferase may be a variant glucosyl transferase. Additionally, the host may express one or more accessory enzymes, proteins, peptides.

Vectors

A DNA construct comprising a nucleic acid encoding a glucosyl transferase can be constructed to be expressed in a host cell. Because of the well-known degeneracy in the genetic code, variant polynucleotides that encode an identical amino acid sequence can be designed and made with routine skill. It is also well-known in the art to optimize codon use for a particular host cell. Nucleic acids encoding glucosyl transferase can be incorporated into a vector. Vectors can be transferred to a host cell using well-known transformation techniques, such as those disclosed below.

The vector may be any vector that can be transformed into and replicated within a host cell. For example, a vector comprising a nucleic acid encoding a glucosyl transferase can be transformed and replicated in a bacterial host cell as a means of propagating and amplifying the vector. The vector also may be transformed into an expression host, so that the encoding nucleic acids can be expressed as a functional glucosyl transferase. Host cells that serve as expression hosts can include filamentous fungi, for example.

A nucleic acid encoding a glucosyl transferase can be operably linked to a suitable promoter, which allows transcription in the host cell. The promoter may be any DNA sequence

that shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Exemplary promoters for directing the transcription of the DNA sequence encoding a glucosyl transferase, especially in a bacterial host, are the promoter of the lac operon of *E. coli*, the *Streptomyces coelicolor* agarase gene dagA or celA promoters, the promoters of the *Bacillus licheniformis* α-amylase gene (amyL), the promoters of the *Bacillus stearothermophilus* maltogenic amylase gene (amyM), the promoters of the *Bacillus amyloliquefaciens* α-amylase (amyQ), the promoters of the *Bacillus subtilis* xylA and xylB genes *etc.* For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding *Aspergillus oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *Aspergillus niger* neutral α-amylase, *A. niger* acid stable α-amylase, *A. niger* glucoamylase, *Rhizomucor miehei* lipase, *A. oryzae* alkaline protease, *A. oryzae* triose phosphate isomerase, or *A. nidulans* acetamidase. When a gene encoding a glucosyl transferaseis expressed in a bacterial species such as *E. coli*, a suitable promoter can be selected, for example, from a bacteriophage promoter including a T7 promoter and a phage lambda promoter. Examples of suitable promoters for the expression in a yeast species include but are not limited to the Gal 1 and Gal 10 promoters of *Saccharomyces cerevisiae* and the *Pichia pastoris* AOX1 or AOX2 promoters. *cbh1* is an endogenous, inducible promoter from *T. reesei*. *See* Liu *et al.* (2008) "Improved heterologous gene expression in *Trichoderma reesei* by cellobiohydrolase I gene (cbh1) promoter optimization," *Acta Biochim. Biophys. Sin (Shanghai)* 40(2): 158-65.

The coding sequence can be operably linked to a signal sequence. The DNA encoding the signal sequence may be the DNA sequence naturally associated with the glucosyl transferase gene to be expressed or from a different Genus or species. A signal sequence and a promoter sequence comprising a DNA construct or vector can be introduced into a fungal host cell and can be derived from the same source. For example, the signal sequence is the *cbh1* signal sequence that is operably linked to a *cbh1* promoter.

An expression vector may also comprise a suitable transcription terminator and, in eukaryotes, polyadenylation sequences operably linked to the DNA sequence encoding a variant glucosyl transferase. Termination and polyadenylation sequences may suitably be derived from the same sources as the promoter.

The vector may further comprise a DNA sequence enabling the vector to replicate in the host cell. Examples of such sequences are the origins of replication of plasmids pUC19, pACYC177, pUB110, pE194, pAMB1, and pIJ702.

The vector may also comprise a selectable marker, *e.g.*, a gene the product of which complements a defect in the isolated host cell, such as the *dal* genes from *B. subtilis* or *B. licheniformis*, or a gene that confers antibiotic resistance such as, *e.g.*, ampicillin, kanamycin, chloramphenicol, or tetracycline resistance. Furthermore, the vector may comprise *Aspergillus* selection markers such as *amdS*, *argB*, *niaD* and xx*sC*, a marker giving rise to hygromycin resistance, or the selection may be accomplished by co-transformation, such as known in the art. *See e.g.,* International PCT Application WO 91/17243.

Intracellular expression may be advantageous in some respects, *e.g.*, when using certain bacteria or fungi as host cells to produce large amounts of glucosyl transferase for subsequent enrichment or purification. Extracellular secretion of glucosyl transferase into the culture medium can also be used to make a cultured cell material comprising the isolated glucosyl transferase.

The expression vector typically includes the components of a cloning vector, such as, for example, an element that permits autonomous replication of the vector in the selected host organism and one or more phenotypically detectable markers for selection purposes. The expression vector normally comprises control nucleotide sequences such as a promoter, operator, ribosome binding site, translation initiation signal and optionally, a repressor gene or one or more activator genes. Additionally, the expression vector may comprise a sequence coding for an amino acid sequence capable of targeting the glucosyl transferaseto a host cell organelle such as a peroxisome, or to a particular host cell compartment. Such a targeting sequence includes but is not limited to the sequence, SKL. For expression under the direction of control sequences, the nucleic acid sequence of the glucosyl transferase is operably linked to the control sequences in proper manner with respect to expression.

The procedures used to ligate the DNA construct encoding a glucosyl transferase, the promoter, terminator and other elements, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (see, *e.g.,* Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed., Cold Spring Harbor, 1989, and 3rd ed., 2001).

Transformation and Culture of Host Cells

An isolated cell, either comprising a DNA construct or an expression vector, is advantageously used as a host cell in the recombinant production of a glucosyl transferase. The cell may be transformed with the DNA construct encoding the enzyme, conveniently by integrating the DNA construct (in one or more copies) in the host chromosome. This integration is generally considered to be an advantage, as the DNA sequence is more likely to be stably maintained in the cell. Integration of the DNA constructs into the host chromosome may be performed according to conventional methods, *e.g.*, by homologous or heterologous recombination. Alternatively, the cell may be transformed with an expression vector as described above in connection with the different types of host cells.

Examples of suitable bacterial host organisms are Gram positive bacterial species such as *Bacillaceae* including *Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Geobacillus* (formerly *Bacillus*) *stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus lautus, Bacillus megaterium,* and *Bacillus thuringiensis*; *Streptomyces* species such as *Streptomyces murinus*; lactic acid bacterial species including *Lactococcus* sp. such as *Lactococcus lactis*; *Lactobacillus* sp. including *Lactobacillus reuteri*; *Leuconostoc* sp.; *Pediococcus* sp.; and *Streptococcus* sp. Alternatively, strains of a Gram negative bacterial species belonging to *Enterobacteriaceae* including *E. coli*, or to *Pseudomonadaceae* can be selected as the host organism.

A suitable yeast host organism can be selected from the biotechnologically relevant yeasts species such as but not limited to yeast species such as *Pichia* sp., *Hansenula* sp., or *Kluyveromyces*, *Yarrowinia, Schizosaccharomyces* species or a species of *Saccharomyces,* including *Saccharomyces cerevisiae* or a species belonging to *Schizosaccharomyces* such as, for example, *S. pombe* species. A strain of the methylotrophic yeast species, *Pichia pastoris*, can be used as the host organism. Alternatively, the host organism can be a *Hansenula* species. Suitable host organisms among filamentous fungi include species of *Aspergillus, e.g., Aspergillus niger, Aspergillus oryzae, Aspergillus tubigensis, Aspergillus awamori*, or *Aspergillus nidulans*. Alternatively, strains of a *Fusarium* species, *e.g., Fusarium oxysporum* or of a *Rhizomucor* species such as *Rhizomucor miehei* can be used as the host organism. Other suitable strains include *Thermomyces* and *Mucor* species. In addition, *Trichoderma sp.* can be used as a host. A suitable procedure for transformation of *Aspergillus* host cells includes, for

example, that described in EP 238023. A glucosyl transferase expressed by a fungal host cell can be glycosylated, *i.e.*, will comprise a glycosyl moiety. The glycosylation pattern can be the same or different as present in the wild-type glucosyl transferase. The type and/or degree of glycosylation may impart changes in enzymatic and/or biochemical properties.

It is advantageous to delete genes from expression hosts, where the gene deficiency can be cured by the transformed expression vector. Known methods may be used to obtain a fungal host cell having one or more inactivated genes. Gene inactivation may be accomplished by complete or partial deletion, by insertional inactivation or by any other means that renders a gene nonfunctional for its intended purpose, such that the gene is prevented from expression of a functional protein. A gene from a *Trichoderma* sp. or other filamentous fungal host that has been cloned can be deleted, for example, *cbh1*, *cbh2*, *egl1*, and *egl2* genes. Gene deletion may be accomplished by inserting a form of the desired gene to be inactivated into a plasmid by methods known in the art.

Introduction of a DNA construct or vector into a host cell includes techniques such as transformation; electroporation; nuclear microinjection; transduction; transfection, *e.g.*, lipofection mediated and DEAE-Dextrin mediated transfection; incubation with calcium phosphate DNA precipitate; high velocity bombardment with DNA-coated microprojectiles; and protoplast fusion. General transformation techniques are known in the art. *See, e.g.*, Sambrook *et al.* (2001), *supra*. The expression of heterologous protein in *Trichoderma* is described, for example, in U.S. Patent No. 6,022,725. Reference is also made to Cao *et al.* (2000) *Science* 9:991-1001 for transformation of *Aspergillus* strains. Genetically stable transformants can be constructed with vector systems whereby the nucleic acid encoding a glucosyl transferase is stably integrated into a host cell chromosome. Transformants are then selected and purified by known techniques.

Expression

A method of producing a glucosyl transferase may comprise cultivating a host cell as described above under conditions conducive to the production of the enzyme and recovering the enzyme from the cells and/or culture medium.

The medium used to cultivate the cells may be any conventional medium suitable for growing the host cell in question and obtaining expression of a glucosyl transferase. Suitable media and media components are available from commercial suppliers or may be prepared

according to published recipes (*e.g.*, as described in catalogues of the American Type Culture Collection).

An enzyme secreted from the host cells can be used in a whole broth preparation. In the present methods, the preparation of a spent whole fermentation broth of a recombinant microorganism can be achieved using any cultivation method known in the art resulting in the expression of a glucosyl transferase. Fermentation may, therefore, be understood as comprising shake flask cultivation, small- or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermenters performed in a suitable medium and under conditions allowing the glucosyl transferase to be expressed or isolated. The term "spent whole fermentation broth" is defined herein as unfractionated contents of fermentation material that includes culture medium, extracellular proteins (*e.g.*, enzymes), and cellular biomass. It is understood that the term "spent whole fermentation broth" also encompasses cellular biomass that has been lysed or permeabilized using methods well known in the art.

An enzyme secreted from the host cells may conveniently be recovered from the culture medium by well-known procedures, including separating the cells from the medium by centrifugation or filtration, and precipitating proteinaceous components of the medium by means of a salt such as ammonium sulfate, followed by the use of chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

The polynucleotide encoding a glucosyl transferase in a vector can be operably linked to a control sequence that is capable of providing for the expression of the coding sequence by the host cell, *i.e.* the vector is an expression vector. The control sequences may be modified, for example by the addition of further transcriptional regulatory elements to make the level of transcription directed by the control sequences more responsive to transcriptional modulators. The control sequences may in particular comprise promoters.

Host cells may be cultured under suitable conditions that allow expression of a glucosyl transferase. Expression of the enzymes may be constitutive such that they are continually produced, or inducible, requiring a stimulus to initiate expression. In the case of inducible expression, protein production can be initiated when required by, for example, addition of an inducer substance to the culture medium, for example dexamethasone or IPTG or Sophorose.

Polypeptides can also be produced recombinantly in an *in vitro* cell-free system, such as the TNT™ (Promega) rabbit reticulocyte system.

Methods for Enriching and Purifying glucosyl transferases

Fermentation, separation, and concentration techniques are well known in the art and conventional methods can be used in order to prepare a glucosyl transferase polypeptide-containing solution.

After fermentation, a fermentation broth is obtained, the microbial cells and various suspended solids, including residual raw fermentation materials, are removed by conventional separation techniques in order to obtain a glucosyl transferase solution. Filtration, centrifugation, microfiltration, rotary vacuum drum filtration, ultrafiltration, centrifugation followed by ultra-filtration, extraction, or chromatography, or the like, are generally used.

It is desirable to concentrate a glucosyl transferase polypeptide-containing solution in order to optimize recovery. Use of unconcentrated solutions requires increased incubation time in order to collect the enriched or purified enzyme precipitate.

The enzyme containing solution is concentrated using conventional concentration techniques until the desired enzyme level is obtained. Concentration of the enzyme containing solution may be achieved by any of the techniques discussed herein. Exemplary methods of enrichment and purification include but are not limited to rotary drum vacuum filtration and/or ultrafiltration.

GTF Enzymes

Glucan polymers produced by adding a GTF enzyme to an appropriate solution of sucrose can be soluble or insoluble. Solubility of glucan depends on a number of factors, including percent of alpha 1,3 linkages, percent of alpha 1,6 linkages and polymer length (DP_n). See, e.g., U.S. Patent No. 8,871,474, incorporated herein by reference in its entirety (the '474 patent).

Other products (byproducts) of GTF include glucose (where glucose is hydrolyzed from the glucosyl-GTF enzyme intermediate complex), various soluble oligosaccharides (DP2-DP7), and leucrose (where glucose of the glucosyl-gtf enzyme intermediate complex is linked to fructose). Leucrose is a disaccharide composed of glucose and fructose linked by an alpha-1,5 linkage. Wild type forms of glucosyl transferase enzymes generally contain (in the N-terminal to

C-terminal direction) a signal peptide, a variable domain, a catalytic domain, and a glucan-binding domain.

The glucosyl transferases in certain embodiments of the invention may be derived from a *Streptococcus* species, *Leuconostoc* species or *Lactobacillus* species, for example. Examples of *Streptococcus* species from which the glucosyl transferase may be derived include *S. salivarius*, *S. sobrinus*, *S. dentirousetti*, *S. downei*, *S. mutans*, *S. oralis*, *S. gallolyticus* and *S. sanguinis*. Examples of Leuconostoc species from which the glucosyl transferase may be derived include *L. mesenteroides*, *L. amelibiosum*, *L. argentinum*, *L. carnosum*, *L. citreum*, *L. cremoris*, *L. dextranicum* and *L. fructosum*. Examples of Lactobacillus species from which the glucosyl transferase may be derived include *L. acidophilus*, *L. delbrueckii*, *L. helveticus*, *L. salivarius*, *L. casei*, *L. curvatus*, *L. plantarum*, *L. sakei*, *L. brevis*, *L. buchneri*, *L. fermentum* and *L. reuteri*.

In accordance with an aspect of the present invention, it has been determined that GTF enzymes producing insoluble glucan are particularly preferred. Insoluble glucan is glucan which is not soluble in aqueous solutions. As set forth in the '474 patent, insoluble glucan polymers tend to have a relatively high percentage of alpha 1,3 linkages to alpha 1,6 linkages and a DP_n of at least 100. In accordance with an aspect of the present invention, the following GTF enzymes can be used to form insoluble glucan polymers: GTFJ, GTF300, GTF0874, 6855, 2379, 7527, 1724, 0544, 5926, 4297, 5618, 2765, 0427, 2919, 2678, and 3929.

SEQ ID NO:	Sequene	Origin
1	MDETQDKTVTQSNSGTTASLVTSPEATKEADKRTNTKEADVLTPAKETNAVETATTTNTQ ATAEAATTATTADVAVAAVPNKEAVVTTDAPAVTTEKAEEQPATVKAEVVNTEVKAPEAA LKDSEVEAALSLKNIKNIDGKYYYVNEDGSHKENFAITVNGQLLYFGKDGALTSSSTYSF TPGTTNIVDGFSINNRAYDSSEASFELIDGYLTADSWYRPASIIKDGVTWQASTAEDFRP LLMAWWPNVDTQVNYLNYMSKVFNLDAKYSSTDKQETLKVAAKDIQIKIEQKIQAEKSTQ WLRETISAFVKTQPQWNKETENYSKGGGEDHLQGGALLYVNDSRTPWANSDYRRLNRTAT NQTGTIDKSILDEQSDPNHMGGFDFLLANDVDLSNPVVQAEQLNQIHYLMNWGSIVMGDK DANFDGIRVDAVDNVDADMLQLYTNYFREYYGVNKSEANALAHISVLEAWSLNDNHYNDK TDGAALAMENKQRLALLFSLAKPIKERTPAVSPLYNNTFNTTQRDEKTDWINKDGSKAYN EDGTVKQSTIGKYNEKYGDASGNYVFIRAHDNNVQDIIAEIIKKEINPKSDGFTITDAEM KQAFEIYNKDMLSSDKKYTLNNIPAAYAVMLQNMETITRVYYGDLYTDDGHYMETKSPYY DTIVNLMKSRIKYVSGGQAQRSYWLPTDGKMDNSDVELYRTNEVYTSVRYGKDIMTANDT EGSKYSRTSGQVTLVANNPKLNLDQSAKLNVEMGKIHANQKYRALIVGTADGIKNFTSDA DAIAAGYVKETDSNGVLTFGANDIKGYETFDMSGFVAVWVPVGASDNQDIRVAPSTEAKK EGELTLKATEAYDSQLIYEGFSNFQTIPDGSDPSVYTNRKIAENVDLFKSWGVTSFEMAP QFVSADDGTFLDSVIQNGYAFADRYDLAMSKNNKYGSKEDLRDALKALHKAGIQAIADWV PDQIYQLPGKEVVTATRTDGAGRKIADAIIDHSLYVANSKSSGKDYQAKYGGEFLAELKA KYPEMFKVNMISTGKPIDDSVKLKQWKAEYFNGTNVLERGVGYVLSDEATGKYFTVTKEG NFIPLQLTGKEKVITGFSSDGKGITYFGTSGTQAKSAFVTFNGNTYYFDARGHMVTNSEY	Unknown Streptococcus species

	SPNGKDVYRFLPNGIMLSNAFYIDANGNTYLYNSKGQMYKGGYTKFDVSETDKDGKESKV VKFRYFTNEGVMAKGVTVIDGFTQYFGEDGFQAKDKLVTFKGKTYYFDAHTGNGIKDTWR NINGKWYYFDANGVAATGAQVINGQKLYFNEDGSQVKGGVVKNADGTYSKYKEGFGELVT NEFFTTDGNVWYYAGANGKTVTGAQVINGQHLYFNADGSQVKGGVVKNADGTYSKYNAST GERLTNEFFTTGDNNWYYIGANGKSVTGEVKIGDDTYFFAKDGKQVKGQTVSAGNGRISY YYGDSGKRAVSTWIEIQPGVYVYFDKNGLAYPPRVLN	
2	MIDGKYYYVNEDGSHKENFAITVNGQLLYFGKDGALTSSSTYSFTPGTTNIVDGFSINNR AYDSSEASFELIDGYLTADSWYRPASIIKDGVTWQASTAEDFRPLLMAWWPNVDTQVNYL NYMSKVFNLDAKYSSTDKQETLKVAAKDIQIKIEQKIQAEKSTQWLRETISAFVKTQPQW NKETENYSKGGGEDHLQGGALLYVNDSRTPWANSDYRRLNRTATNQTGTIDKSILDEQSD PNHMGGFDFLLANDVDLSNPVVQAEQLNQIHYLMNWGSIVMGDKDANFDGIRVDAVDNVD ADMLQLYTNYFREYYGVNKSEANALAHISVLEDWSLNDNHYNDKTDGAALAMENKQRLAL LFSLAKPIKERTPAVSPLYNNTFNTTQRDEKTDWINKDGSKAYNEDGTVKQSTIGKYNEK YGDASGNYVYIRAHDNNVQDIIAEIIKKEINPKSDGFTITDAEMKQAFEIYNKDMLSSDK KYTLNNIPAAYAVMLQNMETITRVYYGDLYTDDGHYMETKSPYYDTIVNLMKSRIKYVSG GQAQRSYWLPTDGKMDNSDVELYSTNEVYTSVRYGKDIMTANDTEGSKYSRTSGQVTLVA NNPKLTLDQSAKLNVEMGKIHANQKYRALIVGTADGIKNFTSDADAIAAGYVKETDSNGV LTFGANDIKGYETFDMSGFVAVWVPVGASDDQDIRVAPSTEAKKEGELTLKATEAYDSQL IYEGFSNFQTIPDGSDPSVYTNRKIAENVDLFKSWGVTSFEMAPQFVSADGGTFLDSVIQ NGYAFADRYDLAMSKNNKYGSKEDLRDALKALHKAGIQAIADWVPDQIYQLPGKEVVTAT RTDGAGRKIADAIIDHSLYVANTKSSGKDYQAKYGGEFLAELKAKYPEMFKVNMISTGKP IDDSVKLKQWKAEYFNGTNVLERGVGYVLSDEATGKYFTVTKDGNFIPLQLTGNEKVVTG FSNDGKGITYFGTSGTQAKSAFVTFNGNTYYFDARGHMVTNGEYSPNGKDVYRFLPNGIM LSNAFYVDANGNTYLYNSKGQMYKGGYTKFDVTETDKDGKESKVVKFRYFTNEGVMAKGV TVIDGFTQYFGEDGFQAKDKLVTFKGKTYYFDAHTGNAIKDTWRNINGKWYHFDANGVAA TGAQVINGQKLYFNEDGSQVKGGVVKNADGTYSKYKEGSGELVTNEFFTTDGNVWYYAGA NGKTVTGAQVINGQHLYFNADGSQVKGGVVKNADGTYSKYDASTGERLTNEFFTTGDNNW YYIGANGKSVTGEVKIGDDTYFFAKDGKQVKGQTVSAGNGRISYYYGDSGKRAVSTWIEI QPGVYVYFDKNGIAYPPRVLN	Unknown Streptococcus species
3	MVDGKYYYYDQDGNVKKNFAVSVGDKIYYFDETGAYKDTSKVDADKSSSAVSQNATIFAA NNRAYSTSAKNFEAVDNYLTADSWYRPKSILKDGKTWTESGKDDFRPLLMAWWPDTETKR NYVNYMNKVVGIDKTYTAETSQADLTAAAELVQARIEQKITSENNTKWLREAISAFVKTQ PQWNGESEKPYDDHLQNGALLFDNQTDLTPDTQSNYRLLNRTPTNQTGSLDSRFTYNPND PLGGYDFLLANDVDNSNPVVQAEQLNWLHYLLNFGSIYANDADANFDSIRVDAVDNVDAD LLQISSDYLKAAYGIDKNNKNANNHVSIVEAWSDNDTPYLHDDGDNLMNMDNKFRLSMLW SLAKPLDKRSGLNPLIHNSLVDREVDDREVETVPSYSFARAHDSEVQDIIRDIIKAEINP NSFGYSFTQEEIEQAFKIYNEDLKKTDKKYTHYNVPLSYTLLLTNKGSIPRVYYGDMFTD DGQYMANKTVNYDAIESLLKARMKYVSGGQAMQNYQIGNGEILTSVRYGKGALKQSDKGD ATTRTSGVGVVMGNQPNFSLDGKVVALNMGAAHANQEYRALMVSTKDGVATYATDADASK AGLVKRTDENGYLYFLNDDLKGVANPQVSGFLQVWVPVGAADDQDIRVAASDTASTDGKS LHQDAAMDSRVMFEGFSNFQSFATKEEEYTNVVIANNVDKFVSWGITDFEMAPQYVSSTD GQFLDSVIQNGYAFTDRYDLGMSKANKYGTADQLVKAIKALHAKGLKVMADWVPDQMYTF PKQEVVTVTRTDKFGKPIAGSQINHSLYVTDTKSSGDDYQAKYGGAFLDELKEKYPELFT KKQISTGQAIDPSVKIKQWSAKYFNGSNILGRGADYVLSDQVSNKYFNVASDTLFLPSSL LGKVVESGIRYDGKGYIYNSSATGDQVKASFITEAGNLYYFGKDGYMVTGAQTINGANYF FLENGTALRNTIYTDAQGNSHYYANDGKRYENGYQQFGNDWRYFKDGNMAVGLTTVDGNV QYFDKDGVQAKDKIIVTRDGKVRYFDQHNGNAATNTFIADKTGHWYYLGKDGVAVTGAQT VGKQKLYFEANGQQVKGDFVTSDEGKLYFYDVDSGDMWTDTFIEDKAGNWFYLGKDGAAV TGAQTIRGQKLYFKANGQQVKGDIVKGTDGKIRYYDAKSGEQVFNKTVKAADGKTYVIGN DGVAVDPSVVKGQTFKDASGALRFYNLKGQLVTGSGWYETANHDWVYIQSGKALTGEQTI NGQHLYFKEDGHQVKGQLVTGTDGKVRYYDANSGDQAFNKSVTVNGKTYYFGNDGTAQTA GNPKGQTFKDGSDIRFYSMEGQLVTGSGWYENAQGQWLYVKNGKVLTGLQTVGSQRVYFD ENGIQAKGKAVRTSDGKIRYFDENSGSMITNQWKFVYGQYYYFGNDGARIYRGWN	Streptococcus sobrinus
4	MIDGKYYYVNEDGSHKENFAITVNGQLLYFGKDGALTSSSTYSFTPGTTNIVDGFSINNR AYDSSEASFELIDGYLTADSWYRPASIIKDGVTWQASTAEDFRPLLMAWWPNVDTQVNYL	Streptococcus salivarius

	NYMSKVFNLDAKYSSTDKQETLKVAAKDIQIKIEQKIQAEKSTQWLRETISAFVKTQPQW NKETENYSKGGGEDHLQGGALLYVNDSRTPWANSDYRRLNRTATNQTGTIDKSILDEQSD PNHMGGFDFLLANDVDLSNPVVQAEQLNQIHYLMNWGSIVMGDKDANFDGIRVDAVDNVD ADMLQLYTNYFREYYGVNKSEANALAHISVLEAWSLNDNHYNDKTDGAALAMENKQRLAL LFSLAKPIKERTPAVSPLYNNTFNTTQRDEKTDWINKDGSKAYNEDGTVKQSTIGKYNEK YGDASGNYVFIRAHDNNVQDIIAEIIKKEINPKSDGFTITDAEMKQAFEIYNKDMLSSDK KYTLNNIPAAYAVMLQNMETITRVYYGDLYTDDGHYMETKSPYYDTIVNLMKSRIKYVSG GQAQRSYWLPTDGKMDNSDVELYRTNEVYTSVRYGKDIMTANDTEGSKYSRTSGQVTLVA NNPKLTLDQSAKLNVEMGKIHANQKYRALIVGTADGIKNFTSDADAIAAGYVKETDSNGV LTFGANDIKGYETFDMSGFVAVWVPVGASDDQDIRVAPSTEAKKEGELTLKATEAYDSQL IYEGFSNFQTIPDGSDPSVYTNRKIAENVDLFKSWGVTSFEMAPQFVSADDGTFLDSVIQ NGYAFADRYDLAMSKNNKYGSKEDLRDALKALHKAGIQAIADWVPDQIYQLPGKEVVTAT RTDGAGRKIADAIIDHSLYVANTKSSGKDYQAKYGGEFLAELKAKYPEMFKVNMISTGKP IDDSVKLKQWKAEYFNGTNVLERGVGYVLSDEATGKYFTVTKDGNFIPLQLTGNEKVVTG FSNDGKGITYFGTSGTQAKSAFVTFNGNTYYFDARGHMVTNGEYSPNGKDVYRFLPNGIM LSNAFYVDANGNTYLYNSKGQMYKGGYTKFDVTETDKDGKESKVVKFRYFTNEGVMAKGV TVIDGFTQYFGEDGFQAKDKLVTFKGKTYYFDAHTGNAIKDTWRNINGKWYHFDANGVAA TGAQVINGQKLYFNEDGSQVKGGVVKNADGTYSKYKEGSGELVTNEFFTTDGNVWYYAGA NGKTVTGAQVINGQHLYFNADGSQVKGGVVKNADGTYSKYDASTGERLTNEFFTTGDNNW YYIGANGKSVTGEVKIGDDTYFFAKDGKQVKGQTVSAGNGRISYYYGDSGKRAVSTWIEI QPGVYVYFDKNGIAYPPRVLN	
5	MPSHIKTINGKQYYVEDDGTIRKNYVLERIGGSQYFNAETGELSNQKEYRFDKNGGTGSS ADSTNTNVTVNGDKNAFYGTTDKDIELVDGYFTANTWYRPKEILKDGKEWTASTENDKRP LLTVWWPSKAIQASYLNYMKEQGLGTNQTYTSFSSQTQMDQAALEVQKRIEERIAREGNT DWLRTTIKNFVKTQPGWNSTSENLDNNDHLQGGALLYNNDSRTSHANSDYRLLNRTPTSQ TGKHNPKYTKDTSNGGFEFLLANDIDNSNPAVQAEQLNWLHYIMNIGTITGGSEDENFDG VRVDAVDNVNADLLQIASDYFKAKYGADQSQDQAIKHLSILEAWSHNDAYYNEDTKGAQL PMDDPMHLALVYSLLRPIGNRSGVEPLISNSLNDRSESGKNSKRMANYAFVRAHDSEVQS IIGQIIKNEINPQSTGNTFTLDEMKKAFEIYNKDMRSANKQYTQYNIPSAYALMLTHKDT VPRVYYGDMYTDDGQYMAQKSPYYDAIETLLKGRIRYAAGGQDMKVNYIGYGNTNGWDAA GVLTSVRYGTGANSASDTGTAETRNQGMAVIVSNQPALRLTSNLTINMGAAHRNQAYRPL LLTTNDGVATYLNDSDANGIVKYTDGNGNLTFSANEIRGIRNPQVDGYLAVWVPVGASEN QDVRVAPSKEKNSSGLVYESNAALDSQVIYEGFSNFQDFVQNPSQYTNKKIAENANLFKS WGITSFEFAPQYVSSDDGSFLDSVIQNGYAFTDRYDIGMSKDNKYGSLADLKAALKSLHA VGISAIADWVPDQIYNLPGDEVVTATRVNNYGETKDGAIIDHSLYAAKTRTFGNDYQGKY GGAFLDELKRLYPQIFDRVQISTGKRMTTDEKITQWSAKYMNGTNILDRGSEYVLKNGLN GYYGTNGGKVSLPKVVGSNQSTNGDNQNGDGSGKFEKRLFSVRYRYNNGQYAKNAFIKDN DGNVYYFDNSGRMAVGEKTIDGKQYFFLANGVQLRDGYRQNRRGQVFYYDQNGVLNANGK QDPKPDNNNNASGRNQFVQIGNNVWAYYDGNGKRVTGHQNINGQELFFDNNGVQVKGRTV NENGAIRYYDANSGEMARNRFAEIEPGVWAYFNNDGTAVKGSQNINGQDLYFDQNGRQVK GALANVDGNLRYYDVNSGELYRNRFHEIDGSWYYFDGNGNAVKGMVNINGQNLLFDNNGK QIKGHLVRVNGVVRYFDPNSGEMAVNRWVEVSPGWWVYFDGEGRGQI	Streptococcus salivarius
6	MDETQDKTVTQSNSGTTASLVTSPEATKEADKRTNTKEADVLTPAKETNAVETATTTNTQ ATAEAATTATTADVAVAAVPNKEAVVTTDAPAVTTEKAEEQPATVKAEVVNTEVKAPEAA LKDSEVEAALSLKNIKNIDGKYYYVNEDGSHKENFAITVNGQLLYFGKDGALTSSSTYSF TPGTTNIVDGFSINNRAYDSSEASFELIDGYLTADSWYRPASIIKDGVTWQASTAEDFRP LLMAWWPNVDTQVNYLNYMSKVFNLDAKYSSTDKQETLKVAAKDIQIKIEQKIQAEKSTQ WLRETISAFVKTQPQWNKETENYSKGGGEDHLQGGALLYVNDSRTPWANSDYRRLNRTAT NQTGTIDKSILDEQSDPNHMGGFDFLLANDVDLSNPVVQAEQLNQIHYLMNWGSIVMGDK DANFDGIRVDAVDNVDADMLQLYTNYFREYYGVNKSEANALAHISVLEAWSLNDNHYNDK TDGAALAMENKQRLALLFSLAKPIKERTPAVSPLYNNTFNTTQRDEKTDWINKDGSKAYN EDGTVKQSTIGKYNEKYGDASGNYVFIRAHDNNVQDIIAEIIKKEINPKSDGFTITDAEM KQAFEIYNKDMLSSDKKYTLNNIPAAYAVMLQNMETITRVYYGDLYTDDGHYMETKSPYY DTIVNLMKSRIKYVSGGQAQRSYWLPTDGKMDNSDVELYRTNEVYTSVRYGKDIMTANDT EGSKYSRTSGQVTLVANNPKLNLDQSAKLNVEMGKIHANQKYRALIVGTADGIKNFTSDA DAIAAGYVKETDSNGVLTFGANDIKGYETFDMSGFVAVWVPVGASDNQDIRVAPSTEAKK	Streptococcus salivarius

	EGELTLKATEAYDSQLIYEGFSNFQTIPDGSDPSVYTNRKIAENVDLFKSWGVTSFEMAP QFVSADDGTFLDSVIQNGYAFADRYDLAMSKNNKYGSKEDLRDALKALHKAGIQAIADWV PDQIYQLPGKEVVTATRTDGAGRKIADAIIDHSLYVANSKSSGKDYQAKYGGEFLAELKA KYPEMFKVNMISTGKPIDDSVKLKQWKAEYFNGTNVLERGVGYVLSDEATGKYFTVTKEG NFIPLQLTGKEKVITGFSSDGKGITYFGTSGTQAKSAFVTFNGNTYYFDARGHMVTNSEY SPNGKDVYRFLPNGIMLSNAFYIDANGNTYLYNSKGQMYKGGYTKFDVSETDKDGKESKV VKFRYFTNEGVMAKGVTVIDGFTQYFGEDGFQAKDKLVTFKGKTYYFDAHTGNGIKDTWR NINGKWYYFDANGVAATGAQVINGQKLYFNEDGSQVKGGVVKNADGTYSKYKEGFGELVT NEFFTTDGNVWYYAGANGKTVTGAQVINGQHLYFNADGSQVKGGVVKNADGTYSKYNAST GERLTNEFFTTGDNNWYYIGANGKSVTGEVKIGDDTYFFAKDGKQVKGQTVSAGNGRISY YYGDSGKRAVSTWIEIQPGVYVYFDKNGLAYPPRVLN	
7	MVDGKYYYYDQDGNVKKNFAVSVGEKIYYFDETGAYKDTSKVEADKSGSDISKEETTFAA NNRAYSTSAENFEAIDNYLTADSWYRPKSILKDGKTWTESSKDDFRPLLMAWWPDTETKR NYVNYMNKVVGIDKTYTAETSQADLTAAAELVQARIEQKITTEQNTKWLREAISAFVKTQ PQWNGESEKPYDDHLQNGALKFDNQSDLTPDTQSNYRLLNRTPTNQTGSLDSRFTYNAND PLGGYELLLANDVDNSNPIVQAEQLNWLHYLLNFGTIYAKDADANFDSIRVDAVDNVDAD LLQISSDYLKAAYGIDKNNKNANNHVSIVEAWSDNDTPYLHDDGDNLMNMDNKFRLSMLW SLAKPLDKRSGLNPLIHNSLVDREVDDREVETVPSYSFARAHDSEVQDLIRDIIKAEINP NAFGYSFTQDEIDQAFKIYNEDLKKTDKKYTHYNVPLSYTLLLTNKGSIPRVYYGDMFTD DGQYMANKTVNYDAIESLLKARMKYVAGGQAMQNYQIGNGEILTSVRYGKGALKQSDKGD ATTRTSGVGVVMGNQPNFSLDGKVVALNMGAAHANQEYRALMVSTKDGVATYATDADASK AGLVKRTDENGYLYFLNDDLKGVANPQVSGFLQVWVPVGAADDQDIRVAASDTASTDGKS LHQDAAMDSRVMFEGFSNFQSFATKEEEYTNVVIANNVDKFVSWGITDFEMAPQYVSSTD GQFLDSVIQNGYAFTDRYDLGMSKANKYGTADQLVKAIKALHAKGLKVMADWVPDQMYTF PKQEVVTVTRTDKFGKPIAGSQINHSLYVTDTKSSGDDYQAKYGGAFLDELKEKYPELFT KKQISTGQAIDPSVKIKQWSAKYFNGSNILGRGADYVLSDQASNKYLNVSDDKLFLPKTL LGQVVESGIRFDGTGYVYNSSTTGEKVTDSFITEAGNLYYFGQDGYMVTGAQNIKGSNYY FLANGAALRNTVYTDAQGQNHYYGNDGKRYENGYQQFGNDSWRYFKNGVMALGLTTVDGH VQYFDKDGVQAKDKIIVTRDGKVRYFDQHNGNAVTNTFVADKTGHWYYLGKDGVAVTGAQ TVGKQHLYFEANGQQVKGDFVTAKDGKLYFYDVDSGDMWTNTFIEDKAGNWFYLGKDGAA VTGAQTIKGQKLYFKANGQQVKGDIVKDADGKIRYYDAQTGEQVFNKSVSVNGKTYYFGS DGTAQTQANPKGQTFKDGSGVLRFYNLEGQYVSGSGWYETAEHEWVYVKSGKVLTGAQTI GNQRVYFKDNGHQVKGQLVTGNDGKLRYYDANSGDQAFNKSVTVNGKTYYFGSDGTAQTQ ANPKGQTFKDGSGVLRFYNLEGQYVSGSGWYKNAQGQWLYVKDGKVLTGLQTVGNQKVYF DKNGIQAKGKAVRTSDGKVRYFDENSGSMITNQWKFVYGQYYYFGSDGAAVYRGWN	Streptococcus downei
8	MIDGKYYYYDNNGKVRTNFTLIADGKILHFDETGAYTDTSIDTVNKDIVTTRSNLYKKYN QVYDRSAQSFEHVDHYLTAESWYRPKYILKDGKTWTQSTEKDFRPLLMTWWPSQETQRQY VNFMNAQLGINKTYDDTSNQLQLNIAAATIQAKIEAKITTLKNTDWLRQTISAFVKTQSA WNSDSEKPFDDHLQNGAVLYDNEGKLTPYANSNYRILNRTPTNQTGKKDPRYTADNTIGG YEFLLANDVDNSNPVVQAEQLNWLHFLMNFGNIYANDPDANFDSIRVDAVDNVDADLLQI AGDYLKAAKGIHKNDKAANDHLSILEAWSDNDTPYLHDDGDNMINMDNKLRLSLLFSLAK PLNQRSGMNPLITNSLVNRTDDNAETAAVPSYSFIRAHDSEVQDLIRDIIKAEINPNVVG YSFTMEEIKKAFEIYNKDLLATEKKYTHYNTALSYALLLTNKSSVPRVYYGDMFTDDGQY MAHKTINYEAIETLLKARIKYVSGGQAMRNQQVGNSEIITSVRYGKGALKAMDTGDRTTR TSGVAVIEGNNPSLRLKASDRVVVNMGAAHKNQAYRPLLLTTDNGIKAYHSDQEAAGLVR YTNDRGELIFTAADIKGYANPQVSGYLGVWVPVGAAADQDVRVAASTAPSTDGKSVHQNA ALDSRVMFEGFSNFQAFATKKEEYTNVVIAKNVDKFAEWGVTDFEMAPQYVSSTDGSFLD SVIQNGYAFTDRYDLGISKPNKYGTADDLVKAIKALHSKGIKVMADWVPDQMYALPEKEV VTATRVDKYGTPVAGSQIKNTLYVVDGKSSGKDQQAKYGGAFLEELQAKYPELFARKQIS TGVPMDPSVKIKQWSAKYFNGTNILGRGAGYVLKDQATNTYFNISDNKEINFLPKTLLNQ DSQVGFSYDGKGYVYYSTSGYQAKNTFISEGDKWYYFDNNGYMVTGAQSINGVNYYFLPN GLQLRDAILKNEDGTYAYYGNDGRRYENGYYQFMSGVWRHFNNGEMSVGLTVIDGQVQYF DEMGYQAKGKFVTTADGKIRYFDKQSGNMYRNRFIENEEGKWLYLGEDGAAVTGSQTING QHLYFRANGVQVKGEFVTDRHGRISYYDGNSGDQIRNRFVRNAQGQWFYFDNNGYAVTGA RTINGQHLYFRANGVQVKGEFVTDRHGRISYYDGNSGDQIRNRFVRNAQGQWFYFDNNGY AVTGARTINGQHLYFRANGVQVKGEFVTDRYGRISYYDGNSGDQIRNRFVRNAQGQWFYF	Streptococcus mutans

	DNNGYAVTGARTINGQHLYFRANGVQVKGEFVTDRYGRISYYDANSGERVRIN	
9	MVDGKYYYYDADGNVKKNFAVSVGDAIFYFDETGAYKDTSKVDADKTSSSVNQTTETFAA NNRAYSTAAENFEAIDNYLTADSWYRPKSILKDGTTWTESTKDDFRPLLMAWWPDTETKR NYVNYMNKVVGIDKTYTAETSQADLTAAAELVQARIEQKITSEKNTKWLREAISAFVKTQ PQWNGESEKPYDDHLQNGALKFDNETSLTPDTQSGYRILNRTPTNQTGSLDPRFTFNQND PLGGYEYLLANDVDNSNPVVQAESLNWLHYLLNFGSIYANDPEANFDSIRVDAVDNVDAD LLQISSDYLKSAYKIDKNNKNANDHVSIVEAWSDNDTPYLNDDGDNLMNMDNKFRLSMLW SLAKPTNVRSGLNPLIHNSVVDREVDDREVEATPNYSFARAHDSEVQDLIRDIIKAEINP NSFGYSFTQEEIDQAFKIYNEDLKKTNKKYTHYNVPLSYTLLLTNKGSIPRIYYGDMFTD DGQYMANKTVNYDAIESLLKARMKYVSGGQAMQNYNIGNGEILTSVRYGKGALKQSDKGD KTTRTSGIGVVMGNQSNFSLEGKVVALNMGATHTKQKYRALMVSTETGVAIYNSDEEAEA AGLIKTTDENGYLYFLNDDLKGVANPQVSGFLQVWVPVGAPADQDIRVAATDAASTDGKS LHQDAALDSRVMFEGFSNFQSFATKEEEYTNVVIAKNVDKFVSWGITDFEMAPQYVSSTD GTFLDSVIQNGYAFTDRYDLGMSKANKYGTADQLVAAIKALHAKGLRVMADWVPDQMYTF PKKEVVTVTRTDKFGNPVAGSQINHTLYVTDTKGSGDDYQAKYGGAFLDELKEKYPELFT KKQISTGQAIDPSVKIKQWSAKYFNGSNILGRGANYVLSDQASNKYFNVAEGKVFLPAAM LGKVVESGIRFDGKGYIYNSSTTGEQVKDSFITEAGNLYYFGKDGYMVMGAQNIQGANYY FLANGAALRNSILTDQDGKSHYYANDGKRYENGYYQFGNDSWRYFENGVMAVGLTRVAGH DQYFDKDGIQAKNKIIVTRDGKVRYFDEHNGNAATNTFISDQAGHWYYLGKDGVAVTGAQ TVGKQHLYFEANGQQVKGDFVTAKDGKLYFLDGDSGDMWTDTFVQDKAGHWFYLGKDGAA VTGAQTVRGQKLYFKANGQQVKGDIVKGADGKIRYYDANSGDQVYNRTVKGSDGKTYIIG NDGVAITQTIAKGQTIKDGSVLRFYSMEGQLVTGSGWYSNAKGQWLYVKNGQVLTGLQTV GSQRVYFDANGIQAKGKAVRTSDGKLRYFDANSGSMITNQWKEVNGQYYYFDNNGVAIYR GWN	Streptococcus dentirousetti
10	MIDGKNYYVQDDGTVKKNFAVELNGRILYFDAETGALVDSNEYQFQQGTSSLNNEFSQKN AFYGTTDKDIETVDGYLTADSWYRPKFILKDGKTWTASTETDLRPLLMAWWPDKRTQINY LNYMNQQGLGAGAFENKVEQALLTGASQQVQRKIEEKIGKEGDTKWLRTLMGAFVKTQPN WNIKTESETTGTKKDHLQGGALLYTNNEKSPHADSKFRLLNRTPTSQTGTPKYFIDKSNG GYEFLLANDFDNSNPAVQAEQLNWLHYMMNFGSIVANDPTANFDGVRVDAVDNVNADLLQ IASDYFKSRYKVGESEEEAIKHLSILEAWSDNDPDYNKDTKGAQLAIDNKLRLSLLYSFM RNLSIRSGVEPTITNSLNDRSSEKKNGERMANYIFVRAHDSEVQTVIADIIRENINPNTD GLTFTMDELKQAFKIYNEDMRKADKKYTQFNIPTAHALMLSNKDSITRVYYGDLYTDDGQ YMEKKSPYHDAIDALLRARIKYVAGGQDMKVTYMGVPREADKWSYNGILTSVRYGTGANE ATDEGTAETRTQGMAVIASNNPNLKLNEWDKLQVNMGAAHKNQYYRPVLLTTKDGISRYL TDEEVPQSLWKKTDANGILTFDMNDIAGYSNVQVSGYLAVWVPVGAKADQDARTTASKKK NASGQVYESSAALDSQLIYEGFSNFQDFATRDDQYTNKVIAKNVNLFKEWGVTSFELPPQ YVSSQDGTFLDSIIQNGYAFEDRYDMAMSKNNKYGSLKDLLNALRALHSVNIQAIADWVP DQIYNLPGKEVVTATRVNNYGTYREGAEIKEKLYVANSKTNETDFQGKYGGAFLDELKAK YPEIFERVQISNGQKMTTDEKITKWSAKYFNGTNILGRGAYYVLKDWASNDYLTNRNGEI VLPKQLVNKNSYTGFVSDANGTKFYSTSGYQAKNSFIQDENGNWYYFDKRGYLVTGAHEI DGKHVYFLKNGIQLRDSIREDENGNQYYYDQTGAQVLNRYYTTDGQNWRYFDAKGVMARG LVKIGDGQQFFDENGYQVKGKIVSAKDGKLRYFDKDSGNAVINRFAQGDNPSDWYYFGVE FAKLTGLQKIGQQTLYFDQDGKQVKGKIVTLSDKSIRYFDANSGEMAVGKFAEGAKNEWY YFDKTGKAVTGLQKIGKQTLYFDQDGKQVKGKVVTLADKSIRYFDADSGEMAVGKFAEGA KNEWYYFDQTGKAVTGLQKIDKQTLYFDQDGKQVKGKIVTLSDKSIRYFDANSGEMATNK FVEGSQNEWYYFDQAGKAVTGLQQVGQQTLYFTQDGKQVKGKVVDVNGVSRYFDANSGDM ARSKWIQLEDGSWMYFDRDGRGQNFGRN	Streptococcus oralis
11	MIDGKKYYVQDDGTVKKNFAVELNGKILYFDAETGALIDSAEYQFQQGTSSLNNEFTQKN AFYGTTDKDVETIDGYLTADSWYRPKFILKDGKTWTASTEIDLRPLLMAWWPDKQTQVSY LNYMNQQGLGAGAFENKVEQAILTGASQQVQRKIEERIGKEGDTKWLRTLMGAFVKTQPN WNIKTESETTGTNKDHLQGGALLYSNSDKTSHANSKYRILNRTPTNQTGTPKYFIDKSNG GYEFLLANDFDNSNPAVQAEQLNWLHFMMNFGSIVANDPTANFDGVRVDAVDNVNADLLQ IASDYFKSRYKVGESEEEAIKHLSILEAWSDNDPDYNKDTKGAQLPIDNKLRLSLLYSFM RKLSIRSGVEPTITNSLNDRSTEKKNGERMANYIFVRAHDSEVQTVIADIIRENINPNTD	Streptococcus sanguinis

	GLTFTMDELKQAFKIYNEDMRKADKKYTQFNIPTAHALMLSNKDSITRVYYGDLYTDDGQ YMEKKSPYHDAIDALLRARIKYVAGGQDMKVTYMGVPREADKWSYNGILTSVRYGTGANE ATDEGTAETRTQGMAVIASNNPNLKLNEWDKLQVNMGAAHKNQYYRPVLLTTKDGISRYL TDEEVPQSLWKKTDANGILTFDMNDIAGYSNVQVSGYLAVWVPVGAKADQDARVTASKKK NASGQVYESSAALDSQLIYEGFSNFQDFATRDDQYTNKVIAKNVNLFKEWGVTSFELPPQ YVSSQDGTFLDSIIQNGYAFEDRYDMAMSKNNKYGSLNDLLNALRALHSVNIQAIADWVP DQIYNLPGKEVVTATRVNNYGTYREGSEIKENLYVANTKTNGTDYQGKYGGAFLDELKAK YPEIFERVQISNGQKMTTDEKITKWSAKHFNGTNILGRGAYYVLKDWASNEYLNNKNGEM VLPKQLVNKNAYTGFVSDASGTKYYSTSGYQARNSFIQDENGNWYYFNNRGYLVTGAQEI DGKQLYFLKNGIQLRDSLREDENGNQYYYDKTGAQVLNRYYTTDGQNWRYFDVKGVMARG LVTMGGNQQFFDQNGYQVKGKIARAKDGKLRYFDKDSGNAAANRFAQGDNPSDWYYFGAD GVAVTGLQKVGQQTLYFDQDGKQVKGKVVTLADKSIRYFDANSGEMAVNKFVEGAKNVWY YFDQAGKAVTGLQTINKQVLYFDQDGKQVKGKVVTLADKSIRYFDANSGEMAVGKFAEGA KNEWYYFDQAGKAVTGLQKIGQQTLYFDQNGKQVKGKVVTLADKSIRYFDANSGEMASNK FVEGAKNEWYYFDQAGKAVTGLQQIGQQTLYFDQNGKQVKGKIVYVNGANRYFDANSGEM ARNKWIQLEDGSWMYFDRNGRGRRFGWN	
12	MIDGKYYYVNEDGSHKENFAITVNGQLLYFGKDGALTSSSTYSFTQGTTNIVDGFSINNR AYDSSEASFELIDGYLTADSWYRPASIIKDGVTWQASTAEDFRPLLMAWWPNVDTQVNYL NYMSKVFNLDAKYSSTDKQETLKVAAKDIQIKIEQKIQAEKSTQWLRETISAFVKTQPQW NKETENYSKGGGEDHLQGGALLYVNDSRTPWANSNYRLLNRTATNQTGTIDKSILDEQSD PNHMGGFDFLLANDVDLSNPVVQAEQLNQIHYLMNWGSIVMGDKDANFDGIRVDAVDNVD ADMLQLYTNYFREYYGVNKSEANALAHISVLEAWSLNDNHYNDKTDVAALAMENKQRLAL LFSLAKPIKERTPAVSPLYNNTFNTTQRDEKTDWINKDGSKAYNEDGTVKKSTIGKYNEK YGDASGNYVFIRAHDNNVQDIIAEIIKKEINEKSDGFTITDSEMKRAFEIYNKDMLSNDK KYTLNNIPAAYAVMLQNMETITRVYYGDLYTDDGNYMEAKSPYYDTIVNLMKSRIKYVSG GQAQRSYWLPTDGKMDKSDVELYRTNEVYTSVRYGKDIMTADDTQGSKYSRTSGQVTLVV NNPKLTLDQSAKLNVVMGKIHANQKYRALIVGTPNGIKNFTSDAEAIAAGYVKETDGNGV LTFGANDIKGYETFDMSGFVAVWVPVGASDDQDIRVAASTAAKKEGELTLKATEAYDSQL IYEGFSNFQTIPDGSDPSVYTNRKIAENVDLFKSWGVTSFEMAPQFVSADDGTFLDSVIQ NGYAFADRYDLAMSKNNKYGSKEDLRNALKALHKAGIQAIADWVPDQIYQLPGKEVVTAT RTDGAGRKISDAIIDHSLYVANSKSSGKDYQAKYGGEFLAELKAKYPEMFKVNMISTGKP IDDSVKLKQWKAEYFNGTNVLDRGVGYVLSDEATGKYFTVTKEGNFIPLQLKGNKKVITG FSSDGKGITYFGTSGNQAKSAFVTFNGNTYYFDARGHMVTNGEYSPNGKDVYRFLPNGIM LSNAFYVDGNGNTYLYNSKGQMYKGGYSKFDVTETKDGKESKVVKFRYFTNEGVMAKGVT VVDGFTQYFNEDGIQSKDELVTYNGKTYYFEAHTGNAIKNTWRNIKGKWYHFDANGVAAT GAQVINGQHLYFNEDGSQVKGSIVKNADGTFSKYKDSSGDLVVNEFFTTGDNVWYYAGAN GKTVTGAQVINGQHLFFKEDGSQVKGDFVKNSDGTYSKYDAASGERLTNEFFTTGDNHWY YIGANGKTVTGEVKIGDDTYFFAKDGKQLKGQIVTTRSGRISYYFGDSGKKAISTWVEIQ PGVFVFFDKNGLAYPPENMN	Unknown Streptococcus species
13	MIDGKYYYVNKDGSHKENFAITVNGQLLYFGKDGALTSSSTYSFTQGTTNIVDGFSKNNR AYDSSEASFELIDGYLTADSWYRPVSIIKDGVTWQASTKEDFRPLLMAWWPNVDTQVNYL NYMSKVFNLDAKYTSTDKQVDLNRAAKDIQVKIEQKIQAEKSTQWLREAISAFVKTQPQW NKETENFSKGGGEDHLQGGALLYVNDPRTPWANSNYRLLNRTATNQTGTIDKSVLDEQSD PNHMGGFDFLLANDVDTSNPVVQAEQLNQIHYLMNWGSIVMGDKDANFDGIRVDAVDNVD ADMLQLYTNYFREYYGVNKSEANALAHISVLEAWSLNDNHYNDKTDGAALAMENKQRLAL LFSLAKPIKERTPAVSPLYNNTFNTTQRDEKTDWINKDGSKAYNEDGTVKQSTIGKYNEK YGDASGNYVFIRAHDNNVQDIIAEIIKKEINPKSDGFTITDAEMKKAFEIYNKDMLSSDK KYTLNNIPAAYAVMLQNMETITRVYYGDLYTDDGHYMETKSPYYDTIVNLMKNRIKYVSG GQAQRSYWLPTDGKMDKSDVELYRTNEVYTSVRYGKDIMTADDTQGSKYSRTSGQVTLVV NNPKLSLDKSAKLDVEMGKIHANQKYRALIVGTPNGIKNFTSDAEAIAAGYVKETDGNGV LTFGANDIKGYETFDMSGFVAVWVPVGASDDQDIRVAASTAAKKEGELTLKATEAYDSQL IYEGFSNFQTIPDGSDPSVYTNRKIAENVDLFKSWGVTSFEMAPQFVSADDGTFLDSVIQ NGYAFADRYDLAMSKNNKYGSKEDLRNALKALHKAGIQAIADWVPDQIYQLPGKEVVTAT RTDGAGRKISDAIIDHSLYVANSKSSGKDYQAKYGGEFLAELKAKYPEMFKVNMISTGKP IDDSVKLKQWKAEYFNGTNVLDRGVGYVLSDEATGKYFTVTKEGNFIPLQLKGNEKVITG FSSDGKGITYFGTSGNQAKSAFVTFNGNTYYFDARGHMVTNGEYSPNGKDVYRFLPNGIM	Streptococcus salivarius

	LSNAFYVDGNGNTYLYNSKGQMYKGGYSKFDVTETKDGKESKVVKFRYFTNEGVMAKGVT VVDGFTQYFNEDGIQSKDELVTYNGKTYYFEAHTGNAIKNTWRNIKGKWYHFDANGVAAT GAQVINGQHLYFNEDGSQVKGGVVKNADGTFSKYKDGSGDLVVNEFFTTGDNVWYYAGAN GKTVTGAQVINGQHLFFKEDGSQVKGDFVKNSDGTYSKYDAASGERLTNEFFTTGDNHWY YIGANGKTVTGEVKIGDDTYFFAKDGKQLKGQIVTTRSGRISYYFGDSGKKAISTWVEIQ PGVFVFFDKNGLAYPPENMN	
14	MTDGKYYYVNEDGSHKENFAITVNGQLLYFGKDGALTSSSTHSFTPGTTNIVDGFSINNR AYDSSEASFELINGYLTADSWYRPVSIIKDGVTWQASTAEDFRPLLMAWWPNVDTQVNYL NYMSKVFNLEAKYTSTDKQADLNRAAKDIQVKIEQKIQAEKSTQWLRETISAFVKTQPQW NKETENYSKGGGEDHLQGGALLYVNDSRTPWANSNYRLLNRTATNQTGTINKSVLDEQSD PNHMGGFDFLLANDVDLSNPVVQAEQLNQIHYLMNWGSIVMGDKDANFDGIRVDAVDNVN ADMLQLYTNYFREYYGVNKSEAQALAHISVLEAWSLNDNHYNDKTDGAALAMENKQRLAL LFSLAKPIKDRTPAVSPLYNNTFNTTQRDFKTDWINKDGSTAYNEDGTAKQSTIGKYNEK YGDASGNYVFIRAHDNNVQDIIAEIIKKEINKKSDGFTISDSEMKQAFEIYNKDMLSSNK KYTLNNIPAAYAVMLQNMETITRVYYGDLYTDDGHYMETKSPYHDTIVNLMKNRIKYVSG GQAQRSYWLPTDGKMDNSDVELYRTSEVYTSVRYGKDIMTADDTEGSKYSRTSGQVTLVV NNPKLTLHESAKLNVEMGKIHANQKYRALIVGTADGIKNFTSDAEAIAAGYVKETDSNGV LTFGANDIKGYETFDMSGFVAVWVPVGASDDQDIRVAPSTEAKKEGELTLKATEAYDSQL IYEGFSNFQTIPDGSDPSVYTNRKIAENVDLFKSWGVTSFEMAPQFVSADDGTFLDSVIQ NGYAFADRYDLAMSKNNKYGSKEDLRDALKALHKAGIQAIADWVPDQIYQLPGKEVVTAT RTDGAGRKIADAIIDHSLYVANSKSSGRDYQAQYGGEFLAELKAKYPKMFTENMISTGKP IDDSVKLKQWKAKYFNGTNVLDRGVGYVLSDEATGKYFTVTKEGNFIPLQLTGNEKAVTG FSNDGKGITYFGTSGNQAKSAFVTFNGNTYYFDARGHMVTNGEYSPNGKDVYRFLPNGIM LSNAFYVDANGNTYLYNYKGQMYKGGYTKFDVTETDKDGNESKVVKFRYFTNEGVMAKGL TVIDGSTQYFGEDGFQTKDKLATYKGKTYYFEAHTGNAIKNTWRNIDGKWYHFDENGVAA TGAQVINGQKLYFNEDGSQVKGGVVKNADGTYSKYKEGSGELVTNEFFTTDGNVWYYAGA DGKTVTGAQVINGQHLYFKEDGSQVKGGVVKNADGTYSKYDAATGERLTNEFFTTGDNNW YYIGSNGKTVTGEVKIGADTYYFAKDGKQVKGQTVTAGNGRISYYYGDSGKKAISTWIEI QPGIYVYFDKTGIAYPPRVLN	Streptococcus salivarius
15	MIDGKYYYVNEDGSHKENFAITVNGQLLYFGKDGALTSSSTYSFTPGTTNIVDGFSINNR AYDSSEASFELIDGYLTADSWYRPASIIKDGVTWQASTAEDFRPLLMAWWPNVDTQVNYL NYMSKVFNLDAKYTSTDKQETLNVAAKDIQVKIEQKIQAEKSTQWLRETISAFVKTQPQW NKETENYSKGGGEDHLQGGALLYVNDSRTPWANSNYRLLNHTATNQKGTIDKSVLDEQSD PNHMGGFDFLLANDVDLSNPVVQAEQLNQIHYLMNWGSIVMGDKDANFDGIRVDAVDNVD ADMLQLYTNYFREYYGVNKSEANALAHISVLEAWSLNDNHYNDKTDGAALAMENKQRLAL LFSLAKPIKERTPAVSPLYNNTFNTTQRDEKTDWINKDGSKAYNEDGTVKQSTIGKYNEK YGDASGNYVFIRAHDNNVQDIIAEIIKKEINPKSDGFTITDAEMKKAFEIYNKDMLSSDK KYTLNNIPAAYAVMLQNMETITRVYYGDLYTDNGNYMETKSPYYDTIVNLMKNRIKYVSG GQAQRSYWLPTDGKMDNSDVELYRTNEVYASVRYGKDIMTADDTEGSKYSRTSGQVTLVA NNPKLTLDQSAKLKVEMGKIHANQKYRALIVGTADGIKNFTSDADAIAAGYVKETDSNGV LTFGANDIKGYETFDMSGFVAVWVPVGASDDQDIRVAPSTEAKKEGELTLKATEAYDSQL IYEGFSNFQTIPDGSDPSVYTNRKIAENVDLFKSWGVTSFEMAPQFVSADDGTFLDSVIQ NGYAFADRYDLAMSKNNKYGSKEDLRDALKALHKAGIQAIADWVPDQIYQLPGKEVVTAT RTDGAGRKIADAIIDHSLYVANTKSSGKDYQAKYGGEFLAELKAKYPEMFKVNMISTGKP IDDSVKLKQWKAEYFNGTNVLERGVGYVLSDEATGKYFTVTKDGNFIPLQLTGNEKVVTG FSNDGKGITYFGTSGTQAKSAFVTFNGNTYYFDARGHMVTNGEYSPNGKDVYRFLPNGIM LSNAFYVDANGNTYLYNSKGQMYKGGYTKFDVTETDKDGKESKVVKFRYFTNEGVMAKGV TVIDGFTQYFGEDGFQAKDKLVTFKGKTYYFDAHTGNAIKNTWRNIDGKWYHFDANGVAA TGAQVINGQKLYFNEDGSQVKGGVVKNADGTYSKYKEGSGELVTNEFFTTDGNVWYYAGA NGKTVTGAQVINGQHLYFNADGSQVKGGVVKNADGTYSKYDAATGERLTNEFFTTGDNNW YYIGANGKTVTGEVKIGDDTYYFAKDGKQVKGQTVSAGNGRISYYYGDSGKRAVSTWVEI QPGVYVYFDKNGLAYPPRVLN	Streptococcus salivarius

Preferred Embodiments of the Invention

In accordance with an aspect of the present invention, a method is provided for generating glucose polymers in a dairy product using a glucosyl transferase to provide increased texture. The method provides high, robust, and smooth texture from the formed glucose polymers. As set forth above, in the context of the present invention, texture means thickness and/or mouthfeel. As discussed above, there is wide spread use in the yogurt industry of starch to provide texture in yogurt. The methods of the present invention surprisingly provide an alternative to starch and other stabilizers for adding texture to yogurt.

In accordance with an aspect of the present invention, added sucrose is converted into glucose polymers and fructose. While the glucose polymers increase the texture of the yogurt, the fructose gives the yogurt a fructose sweetness. Fructose enhances palatability and taste of the yogurt in addition to the improved texture.

In some jurisdictions, components such as enzyme potentially require labeling of the final product as having the component as an ingredient. In an aspect of the present invention, the GTF would be considered a processing aid because the milk maybe heated (including pasteurization), inactivating the GTF. Surprisingly, it has been found that the increased texture provided by the instant invention is not destroyed by heating, even up to 95°C for 6 minutes.

In another aspect of the present invention, it was found that the glucose polymers produced in milk at a neutral pH may have a non-uniform or non-homogenous appearance. After inoculation with culture during the fermentation process, the pH drops and it was found that the glucose polymers present during fermentation have a more homogenous, shiny look.

As mentioned above, stabilizers are frequently added to yogurt to increase texture. In addition to increasing expense because of the cost of the ingredient, added stabilizers such as starch require special handling procedures when the yogurt is poured into smaller containers for distribution to consumers. Yogurt manufacturers must typically cool yogurt to 8° C before it can be shipped out for distribution to stores. While it is possible to quickly batch chill yogurt using cooling plates, this is not possible for yogurt having stabilizer. If yogurt with stabilizer is batch chilled to 8° C before filling into individual containers for consumer purchase, the texture provided by the stabilizer will be destroyed during the filling process by shear forces. Texture lost in this way cannot be restored, defeating the entire point of adding stabilizer to begin with.

Stabilizer containing yogurt must be filled into containers at 20 to 25°C. Once the yogurt with stabilizer is filled into containers, it can be cooled to approximately 8° C and shipped. However, cooling in this way is slower and causes delays in shipping and added expense in terms of providing a cooling facility.

In accordance with an aspect of the present invention, it was discovered that the yogurt containing the produced glucose polymers may be cooled to 5° C before filling. This feature allows for substantial costs savings.

In another aspect of the instant invention, the yogurts containing the produced glucose polymers may be combined with stabilizers such as starch or pectin to provide long shelf life, highly stable, increased texture yogurt. Stabilizers may also be used to prevent sedimentation of protein caused by heating of the yogurt at low pH.

The protein and fat content of yogurt can be modified for cost and/or perceived health reasons. For example, fat provides texture and a desirable taste to yogurt. However, for health reasons consumers may prefer low fat or even non-fat yogurt. The glucose polymers produced in accordance with the instant invention can replace the texture lost by reducing or eliminating fat. Increasing yogurt protein content is also a way of increasing texture. However, there is an expense associated with boosting yogurt protein content. In accordance with the present invention, it has been discovered that the glucose polymers of the instant invention can provide texture in place of or in addition to the added protein.

In accordance with an aspect of the present invention, a method is presented for making a yogurt product having improved texture, improved texture being increased thickness and/or mouthfeel, having the steps of: providing milk; adding sucrose to the milk to form sweetened milk; contacting the sweetened milk with a glucosyl transferase to form an insoluble glucose polymer; inoculating with a starter culture; and fermenting to provide the yogurt product having improved texture which is increased thickness and/or increased mouthfeel.

Preferably, the milk is cow's milk. Preferably, the milk is selected from the group consisting of raw milk, pre-pasteurized milk, whole milk, skim milk, reconstituted milk, lactase treated milk, reduced lactose milk, lactose free milk and condensed milk. In other preferred embodiments, the milk is raw milk.

Preferably, the method has the additional steps of homogenizing and pasteurizing the milk. In a preferred aspect of the instant invention, the step of contacting with glucosyl

transferase is performed after the steps of homogenizing and pasteurizing. In yet another preferred embodiment, the step of contacting with glucosyl transferase is performed before the steps of homogenizing and pasteurizing.

Preferably, the sucrose is added to constitute about 0.1 to 12% (w/w). More preferably, the sucrose is added to constitute about 2 to 8% (w/w). In still more preferred embodiments, the sucrose is added to constitute about 4 to 6% (w/w).

Preferably, the glucosyl transferase is an enzyme which has at least 70% sequence identity to an enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15). More preferably, the glucosyl transferase is an enzyme which has at least 80% sequence identity to an enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15). Still more preferably, the glucosyl transferase is an enzyme which has at least 90% sequence identity to an enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2) , GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15). In yet more preferred embodiments, the glucosyl transferase is an enzyme which has at least 95% sequence identity to GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15). In still more preferred embodiments, the glucosyl transferase is selected from the group

consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15). Still more preferably the glucosyl transferase is GTFJ (SEQ ID NO: 1).

Preferably, the glucosyl transferase is present in the milk in an amount from about 0.005 mg per 100 ml milk to 15 mg per 100 ml milk. More preferably, the glucosyltransferase is present in an amount from about 0.03 mg per 100 ml milk to about 12.5 mg per 100 ml milk.

Preferably, the GTFJ is present in an amount from about 0.033 mg per 100 ml milk to about 12.5 mg per 100 ml milk. More preferably, the GTFJ is present in an amount from about 0.3 mg per 100 ml milk to about 5.0 mg per 100 ml milk.

In other preferred embodiments the glucosyl transferase is GTF300 (SEQ ID NO: 2). Preferably, the GTF300 is present in an amount from about 0.033 mg per 100 ml to about 12.5 mg per 100 ml milk. More preferably, the GTF300 is present in an amount from about 0.3 mg per 100 ml milk to about 5 mg per 100 ml milk.

Preferably, the increased texture is increased thickness. Preferably, the thickness is increased by 30% or more as compared with a control sample (no GTF enzyme). More preferably, the thickness is increased by 50% or more. Still more preferably, the thickness is increased by 70% or more. In yet more preferred embodiments, the thickness is increased by 90% or more. More preferably, the thickness is increased by 100% or more. Still more preferably, the thickness is increased by 110% or more. In the most preferred embodiments, the thickness is increased by 120% or more.

In other preferred embodiments, the increased texture is increased mouthfeel. Preferably the mouthfeel is increased by 30% or more as compared with a control sample (no GTF enzyme). More preferably, the mouthfeel is increased by 50% or more. Still more preferably, the mouthfeel is increased by 70% or more. In yet more preferred embodiments, the mouthfeel is increased by 90% or more. Still more preferably, the mouthfeel is increased by 100% or more. In yet more preferred embodiments, the mouthfeel is increased by 110% or more. In the most preferred embodiments, the mouthfeel is increased by 120% or more.

In accordance with an aspect of the present invention, the milk is low fat milk to provide a low fat yogurt. In a more preferred aspect of the present invention the milk is non-fat milk to provide a non-fat yogurt.

In another preferred aspect of the present invention, the protein content of the milk is adjusted to at least about 3% (w/w). More preferably, the protein content of the milk is adjusted to at least about 3.5%. Still more preferably, the protein content of the milk is adjusted to at least about 3.7% (w/w). In other preferred embodiment, the protein content of the milk is adjusted to at least about 3.8 % (w/w). In still more preferred embodiments, the protein content of the milk is adjusted to at least about 3.9 % (w/w). In still other preferred embodiments, the protein content of the milk is adjusted to at least about 4.0 % (w/w).

In another aspect of the invention, the method includes the further steps of cooling the yogurt of to a temperature of between 5 and 10° C to provide a chilled yogurt; and pouring the chilled yogurt into preformed containers. Preferably, the containers provide a single serving of yogurt. The preferred embodiments of this aspect of the present invention are as set forth above.

In another aspect of the present invention, a yogurt is presented which is made according to any of the above methods. Preferably, the yogurt has pectin.

The present disclosure is described in further detail in the following examples, which are not in any way intended to limit the scope of the disclosure as claimed. The attached figures are meant to be considered as integral parts of the specification and description of the disclosure. The following examples are offered to illustrate, but not to limit the claimed disclosure.

EXAMPLES

Example 1 GTFJ

GTFJ is a glucosyl transferase enzyme derived from *Streptococcus salivarius* SK126 having the amino acid sequence as set forth in SEQ ID NO: 1. GTFJ was produced recombinantly in *Bacillus subtilis*.

Example 2 GTF300

GTF300 has the following backbone substitutions relative to GTFJ: A510D:F607Y:R741S:D948G. The amino acid sequence of GTF300 is set forth in SEQ ID NO: 2. GTF300 was also produced in recombinantly in *B. subtilis*.

Example 3: Standard yogurt procedure

Pre-pasteurised (72 °C for 15 s) bulk blended skimmed milk (0.1 % fat) (Arla Foods, Denmark) stored at 4-6 °C was standardized to a desired protein (% w/w), fat (% w/w) and sucrose (% w/w) content by addition of skimmed milk powder (33 % protein, 1.2 % fat, 54 % carbohydrate) from BBA Lactalis (Laval, Mayenne, France), cream (38 % fat) from Arla Foods Denmark), and sucrose (Granulated Sugar 500, Nordic Sugar A/S, Denmark). The standardized milk was then pasteurized and homogenized in a standard plate heat exchange pasteurizer. Homogenization was performed at 65 °C at 200 bar and pasteurization at 95 °C for 6 minutes, and then milk was cooled to 43 °C. The milk was inoculated with a thermophilic starter culture at an inoculation rate of 20 DCU/100 L. Fermentation was followed using the CINAC multichannel pH system (Ysebaert, Frépillon, France), which monitored the pH development every 5 min. Fermentation was conducted until pH 4.60 and the product was cooled on a yogurt plate heat exchanger (SPX Flow Technology, Sussex, UK) and YTRON-ZP shear-pump system (YTRON Process Technology, Bad Endorf, Germany) to 24 °C. The resulting stirred style yogurts were stored at 4-6 °C for further viscosity measurements.

Example 4: Method for measuring apparent viscosity

A rotational rheological test was employed to evaluate the viscosity of the stirred style yogurts. Flow curves were obtained with an Anton Paar MCR302 rheometer (Anton Paar GmbH, Ostfildern, Germany) using the cone plate measurement system. The test method was a controlled shear rate test (CSR), where the shear rate is controlled and the resulting shear stress is measured. The shear rate intervals applied to the samples were 0.1-200 s^{-1}, which defines the up-curve, and the reverse operation explains the down-curve (200-0.1 s^{-1}). The value of the measuring point duration was selected to be at least as long as the value of the reciprocal shear rate, which is valid for the up-curve. The tests were performed under constant temperature of 10 °C, and each sample was analyzed in duplicates. A water bath was connected to the rheometer to ensure isothermal conditions.

From the flow curves the apparent viscosity was assessed, which is appropriate for fluids where the ratio of shear stress to shear rate varies with the shear rate. The apparent viscosity was extracted at either shear rate 10 Hz or 200 Hz. The apparent viscosity extracted at shear rate 10 Hz indicates the "thickness" of the sample. The apparent viscosity extracted at shear rate 200 s^{-1} (200 Hz) is correlated to the sensory perception of "mouthfeel".

Example 5: GTF300 addition at the inoculation step

The texturing effect of GTF300 was investigated in a 4-liter scale set-up yogurt production. Fresh milk was standardized to 4.0 % (w/w) protein and 1.0 % (w/w) fat, 8.0% (w/w) sucrose, which was homogenized and pasteurized as described in example 3. GTF300 [2.5 mg/100 g of milk] was added at the inoculation step as schematically presented in Figure 1. YO-MIX 860, YO-MIX 495, and YO-MIX 465, respectively, were employed as starter cultures (available from DuPont). After 5 and 28 days, respectively, of storage at 5°C the texturing effect of GTF300 was assessed by rotational rheological test as described in example 4. The impact on viscosity of the non-enzymated and GTF300 added yogurt samples for the three different starter culture fermentations are presented in Figure 2A to 2C (5 days) and Figure 3A to 3C (28 days). The addition of GTF300 provided enhanced shear stress values over the entire shear range for all three starter cultures investigated. The addition of 2.5 mg GTF300 per 100 ml of milk resulted in an increased apparent viscosity ($\dot{\gamma}$ = 200 Hz) compared to the control by 103 %, 122 %, 116 % for YM 860, YM 495, and YM 465, respectively, at day 5. The increase in texture was maintained at day 28 and, in fact, increased.

It was determined that GTF300 provides additional texture to that created by the gel network formed by addition of the starter cultures during acidification to pH 4.6. Moreover, the GTF300 provided texture survives the mechanical shear stresses caused by stirring, pumping and cooling of the fermented milk and this texture increase is maintained after 5 days of storage and, moreover, is maintained throughout the shelf life of the yogurt.

Example 6: GTF300 addition prior to heat-treatment and homogenization

The texturing effect of GTF300 was apparent when added at the inoculation step as shown in example 5. It was of interest to investigate whether the addition of GTF300 and the subsequent texture development could be established prior to pasteurization and homogenization of the base milk and maintained after such processing.

Therefore, GTF300 enzyme [3.75 mg per 100 ml of milk] was added to the base milk containing 8% (w/w%) sucrose, followed by an incubation step at 5 °C for 24 hours. Subsequently, the pasteurization and homogenization were performed as described in example 3. The production flow is schematically presented in Figure 4. YO-MIX 860, YO-MIX 495, YO-MIX 465, and YO-MIX 204, respectively, were employed as starter cultures. After 7 and 28 days of shelf life the apparent viscosity was assessed as described in example 4. The resulting flow curves of the non-enzymated and GTF300 treated samples are shown in Figure 5A – D (7

days) and Figure 6A – D (28 days). The addition of GTF300 increased the apparent viscosity ($\dot{\gamma}$ = 200 Hz) by 72 %, 47 %, 62 %, and 51 % for YO-MIX 860, YO-MIX 495, YO-MIX 465 and YO-MIX 204, respectively, at day 7. This increase in texture was maintained at day 28, see Figure 6A – D (28 days).

Surprisingly, it was observed that the texturing effect established by GTF300 could resist the mechanical shear of homogenization and pasteurization processes. The texture formed during the incubation step is, thus, able to withstand the before mentioned processing steps and in addition the shear from the cooling process at the end of fermentation.

Example 7: GTF300 addition to 2 % and 4 % sucrose yogurts

In example 6 the texturing effect of GTF300 was investigated for yogurts with a content of 8 % sucrose. This prompted investigation of the texturing effect of GTF300 in yogurts with lower sucrose contents. Therefore, the performance of GTF300 was investigated for yogurts with 2 % and 4 % sucrose.

The milk was standardized to 4 % (w/w) protein, 1 % (w/w) fat, and 2 % or 4 % (w/w) sucrose, respectively, and pasteurized and homogenized as described in example 3. The dose of GTF300 was the same with regard to the sucrose content as in example 6. Additionally, doubling the dosage was also investigated. The addition of GTF300 was added to the milk followed by an incubation step at 5 °C for 24 hours prior to pasteurization and homogenization as outlined in Figure 4. The texturing performance of GTF300 was investigated as described in example 4, and the results for day 7 are presented in Figure 7A to 7B.

The texturing effect of GTF300 was apparent for both sucrose contents at 2 % and 4 %. The addition of GTF300 increased the apparent viscosity ($\dot{\gamma}$ = 200 Hz) by 74 % and 61 % when added at 1.88 % sucrose and 3.76 % sucrose for yogurts with 4 % sucrose. For yogurts with 2 % sucrose GTF300 increased the apparent viscosity ($\dot{\gamma}$ = 200 Hz) by 15 % and 30 % when added at 1.88 % sucrose and 3.76 % sucrose, respectively.

Even at reduced sucrose levels a substantial increase in texture can be enabled by the addition of GTF300.

Example 8: Cooling of GTF300 yogurt to 5 °C instead of 24 °C

In the yogurt industry, the cooling of stirred type yogurt containing stabilizers such as starch is performed in a two-phase way. First, the fermented milk is stirred gently to obtain a homogenous matrix, and then cooled to typically between 20-24 °C. Yogurt cups are then filled

and kept at cold storage over a period of 10-12 hours to be cooled below 8 °C. Filling the yogurt cups with yogurt at a temperature between 20-24 °C and then cooling is crucial to maintain the texture added by the starch. In this regard, cooling the yogurt to 8°C and then filling, particularly if the cooling takes place under shear from pumps and plate heat exchanger, could result in a weak yogurt gel. Moreover, whey separation could occur during storage. Therefore, it was of interest to test if the texture formed by GTF300 in the fermented milk could resist cooling to 5 °C and possible shear during cooling and filling.

The milk was standardized to 4 % (w/w) protein, 2 % (w/w) fat, and 8 % (w/w) sucrose and pasteurized and homogenized as described in example 3. The addition of GTF300 [3.75 mg per 100 ml of milk] was added at the inoculation step as schematically presented in Figure 1. The texturing effect of GTF300 in fermented milk cooled to, respectively, 5 °C and 24 °C, when filled in the cups, was assessed after 7 days as described in example 4.

The addition of GTF300 enhanced the apparent viscosity ($\dot{\gamma}$ = 200 Hz) by 89 % and 92 % when cooled at 24 °C and 5 °C, respectively, compared to a non-enzymated yogurt sample cooled at 24 °C. The texture supplied by GTF300 is not sensitive to cooling at 5 °C, and provides the same texture seen for GTF300 yogurts filled at 24 °C (see FIG. 8).

Example 9: Addition of GTF300 to a water model system

The effect of GTF300 was pursued in a water model system with lactose (Variolac® 992 BG100, Arla Foods, Denmark) and/or sucrose (Granulated Sugar 500, Nordic Sugar A/S, Denmark) added. The sucrose and lactose contents were dissolved in the water by stirring the sample on a magnetic stirrer. The samples were kept at 5 °C until analysis of viscosity.

After 24 hours at 5 °C the viscosity was assessed by measuring the Brookfield viscosity (spindle S62, 30 rpm, 30 seconds).

Table 1. Brookfield viscosity of model systems with GTF300 at a dose of 2.5 mg per 100 ml of milk. Brookfield viscosity (spindle S61 or spindle S62, 30 rpm, 30 seconds) was assessed after 24 hours at 5 °C.

Sample	**Brookfield viscosity (cP)**
Sample 1: 5 % lactose + GTF300 2.5 mg/100 ml of milk	2 cP
Sample 2: 5 % lactose + 8 % sucrose + GTF300 2.5 mg/100 ml of milk	200 cP

Sample 3: 8 % sucrose + GTF300 2.5 mg/100 ml of milk	70 cP

As shown above, with no sucrose GTF300 is unable to make polymer. As expected, glucan polymer is formed by the inclusion of 8% sucrose in the aqueous media. Surprisingly, however, it was determined that formation of glucan was substantially increased in the presence of lactose.

Example 10: GTFJ addition at the inoculation step

The texturing effect of GTFJ was investigated in a 4-liter scale set-up yogurt production. Fresh milk and cream was standardized to 4.0 % (w/w) protein and 1.0 % (w/w) fat, 8% (w/w) sucrose, homogenized and pasteurized as described in example 3. GTFJ was added at the inoculation step in several dosages (v/w%) [0.33 mg per 100 ml milk, 0.66 mg per 100 ml milk, 0.98 mg per 100 ml milk, 1.31 mg per 100 ml milk].

The employed starter culture was YO-MIX 860. After 7 days of storage the texturing effect of GTFJ was assessed by rotational rheological test as described in example 4. The results of the non-enzymated and GTFJ added yogurt samples for day 7 are presented in Figure 9A. The addition of GTFJ enhanced the thickness for all applied dosages. The addition of 0.33 mg per 100 ml milk (0,05% enzyme), 0.66 mg per 100 ml milk (0,1% enzyme), 0.98 mg per 100 ml milk (0,15% enzyme), 1.31 mg per 100 mlmilk.(0,2% enzyme) increased the thickness by 62 %, 92 %, 154 %, and 223 %, respectively. The texturing effect of GTFJ was compared to the texturing effect of protein in Figure 9B. It was seen that an addition of GTFJ [0.98 mg per 100 ml milk/0,15% enzyme] to a 3.7 % protein yogurt increased the shear stress over the entire shear rate range. The flow curve of the 3.7 % protein yogurt sample added GTFJ [0.98 mg per 100 ml milk] was compared to a non-enzymated 4.0 % protein yogurt sample, and it was seen that the addition of GTFJ to a 3.7 % protein yogurt sample could mimic the flow curve of the 4.0 % protein yogurt sample. The apparent viscosity ($\dot{\gamma}$ = 200 Hz) of the 3.7 % non-enzymated yogurt sample was 67 Pa. The addition of GTFJ increased the apparent viscosity ($\dot{\gamma}$ = 200 Hz) by 45 % to 97 Pa. The apparent viscosity ($\dot{\gamma}$ = 200 Hz) of the 4.0 % non-enzymated yogurt sample was 95 Pa.

Although the foregoing invention has been described in some detail by way of illustration and example, for purposes of clarity of understanding, certain changes and modifications can be practiced within the scope of the appended claims. In addition, each reference provided herein is

incorporated by reference in its entirety for all purposes to the same extent as if each reference was individually incorporated by reference. To the extent the content of any citation, including website or accession number may change with time, the version in effect at the filing date of this application is meant. Unless otherwise apparent from the context any step, element, aspect, feature of embodiment can be used in combination with any other.

What is claimed is:

1. A method of making a yogurt product having improved texture, wherein said improved texture comprises increased thickness and/or increased mouthfeel, said method comprising the steps of:

providing milk;

adding sucrose to the milk to form sweetened milk;

contacting said sweetened milk with a glucosyl transferase to form an insoluble glucose polymer;

inoculating with a starter culture;

and fermenting to provide the yogurt product having improved texture comprising increased thickness and/or increased mouthfeel.

2. A method according to claim 1 wherein the milk is cow's milk.

3. A method according to claim 2 wherein the milk is selected from the group consisting of raw milk, pre-pasteurized milk, whole milk, skim milk, reconstituted milk, lactase treated milk, reduced lactose milk, lactose free milk and condensed milk.

4. A method according to claim 3 wherein the milk is raw milk.

5. A method according to any of the preceding claims comprising the additional steps of homogenizing and pasteurizing the milk.

6. A method according to claim 5 wherein the step of contacting with glucosyl transferase is performed after the steps of homogenizing and pasteurizing.

7. A method according to claim 5 wherein the step of contacting with glucosyl transferase is performed before the steps of homogenizing and pasteurizing.

8. A method according to any of the preceding claims wherein the sucrose is added to constitute about 0.1 to 12% (w/w).

9. A method according to claim 8 wherein the sucrose is added to constitute about 2 to 8% (w/w).

10. A method according to claim 9 wherein the sucrose is added to constitute about 4 to 6% (w/w).

11. A method according to any of the preceding claims wherein the glucosyl transferase comprises an enzyme which has at least 70% sequence identity to and enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID

NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15).

12. A method according to claim 11 wherein the glucosyl transferase comprises an enzyme which has at least 80% sequence identity to an enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15).

13. A method according to claim 12 wherein the glucosyl transferase comprises an enzyme which has at least 90% sequence identity to an enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15).

14. A method according to claim 13 wherein the glucosyl transferase comprises an enzyme which has at least 95% sequence identity to an enzyme selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15).

15. A method according to claim 14 wherein the glucosyl transferase is selected from the group consisting of GTFJ (SEQ ID NO: 1), GTF300 (SEQ ID NO: 2), GTF0874 (SEQ ID NO: 3), GTF6855 (SEQ ID NO: 4), GTF2379 (SEQ ID NO: 5), GTF7527 (SEQ ID NO: 6), GTF1724 (SEQ ID NO: 7), GTF0544 (SEQ ID NO: 8), GTF5926 (SEQ ID NO: 9), GTF4297 (SEQ ID NO: 10), GTF5618 (SEQ ID NO: 11), GTF2765 (SEQ ID NO: 12), GTF2919 (SEQ ID NO: 13), GTF2678 (SEQ ID NO; 14), and GTF3929 (SEQ ID NO: 15).

16. A method according to any of claims 11-15 wherein the glucosyl transferase is present in the milk in an amount from about 0.005 mg per 100 ml milk to about 15 mg per 100 ml milk.

17. A method according to claim 16 wherein the glucosyltransferase is present in the milk in an amount from about 0.03 mg per 100 ml milk to about 12.5 mg per 100 ml milk.

18. A method according to claim 15 wherein the glucosyl transferase is GTFJ (SEQ ID NO: 1).

19. A method according to claim 18 wherein the GTFJ is present in an amount from about 0.033 mg per 100 ml milk to about 12.5 mg per 100 ml milk.

20. A method according to claim 19 wherein the GTFJ is present in an amount from about 0.3 mg per 100 ml milk to about 5.0 mg per 100 ml milk.

21. A method according to claim 15 wherein the glucosyl transferase is GTF300 (SEQ ID NO: 2).

22. A method according to claim 21 wherein the GTF300 is present in an amount from about 0.033 mg per 100 ml to about 12.5 mg per 100 ml milk.

23. A method according to claim 22 wherein the GTF300 is present in an amount from about 0.3 mg per100 ml milk to about 5 mg per 100 ml milk.

24. A method according to any of the preceding claims wherein the increased texture comprises increased thickness.

25. A method according to claim 24 wherein the thickness is increased by 30% or more.

26. A method according to claim 25 wherein the thickness is increased by 50% or more.

27. A method according to claim 26 wherein the thickness is increased by 70% or more.

28. A method according to claim 27 wherein the thickness is increased by 90% or more.

29. A method according to claim 28 wherein the thickness is increased by 100% or more.

30. A method according to claim 29 wherein the thickness is increased by 110% or more.

31. A method according to claim 30 wherein the thickness is increased by 120% or more.

32. A method according to any of claims 1-23 wherein the increased texture comprises increased mouthfeel.

33. A method according to claim 32 wherein the mouthfeel is increased by 30% or more.

34. A method according to claim 33 wherein the mouthfeel is increased by 50% or more.

35. A method according to claim 34 wherein the mouthfeel is increased by 70% or more.

36. A method according to claim 35 wherein the mouthfeel is increased by 90% or more.

37. A method according to claim 36 wherein the mouthfeel is increased by 100% or more.

38. A method according to claim 37 wherein the mouthfeel is increased by 110% or more.

39. A method according to claim 38 wherein the mouthfeel is increased by 120% or more.

40. A method according to any of the preceding claims further comprising the steps of

cooling the yogurt of to a temperature of between 5 and 10° C to provide a chilled yogurt; and

pouring the chilled yogurt into preformed containers.

41. A method according to claim 40 wherein the preformed containers provide a single serving of yogurt.

42. A method according to any of the preceding claims wherein the milk is non-fat milk to provide a non-fat yogurt.

43. A method according to any of the preceding claims wherein the protein content of the milk is adjusted to at least about 3% (w/w).

44. A method according to claim 43 wherein the protein content of the milk is adjusted to at least about 3.5% (w/w).

45. A method according to claim 44 wherein the protein content of the milk is adjusted to at least about 3.7% (w/w).

46. A method according to claim 45 wherein the protein content of the milk is adjusted to at least about 3.8 % (w/w).

47. A method according to claim 46 wherein the protein content of the milk is adjusted to at least about 3.9 % (w/w).

48. A method according to claim 47 wherein the protein content of the milk is adjusted to at least about 4.0 % (w/w).

49. A yogurt produced in accordance with any of the preceding claims.

50. The yogurt of claim 49 further comprising pectin.

Fig. 1 - Flow Diagram for Inoculation of Milk and Treatment with GTF enzyme

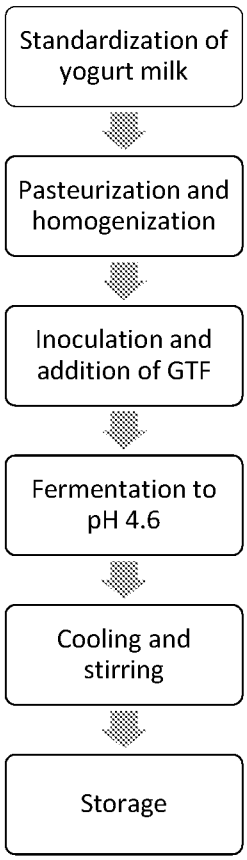


Fig. 2A - (YO-MIX 860) Thickness and mouthfeel as evaluated at 5 days post GTF300 treatment

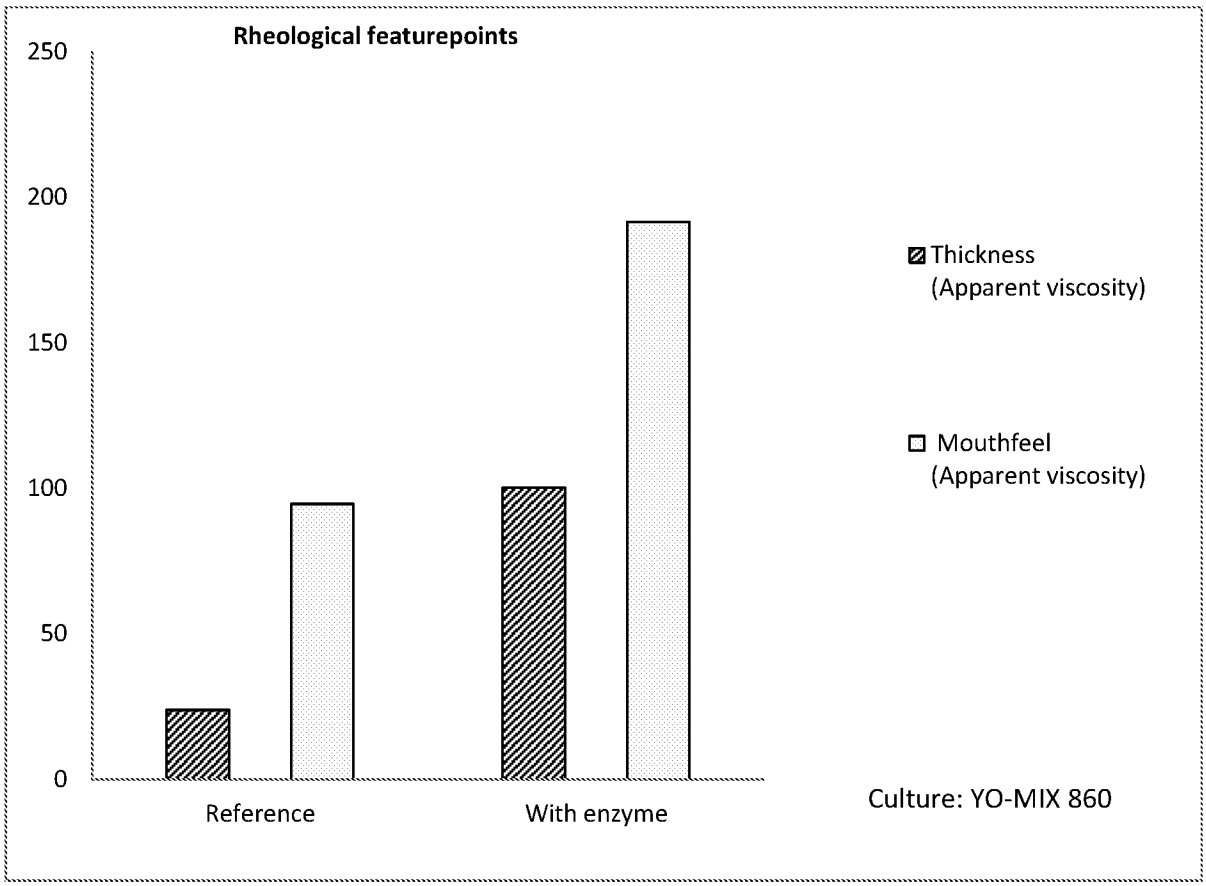


Fig. 2B - (YO-MIX 495) Thickness and mouthfeel as evaluated at 5 days post GTF300 treatment

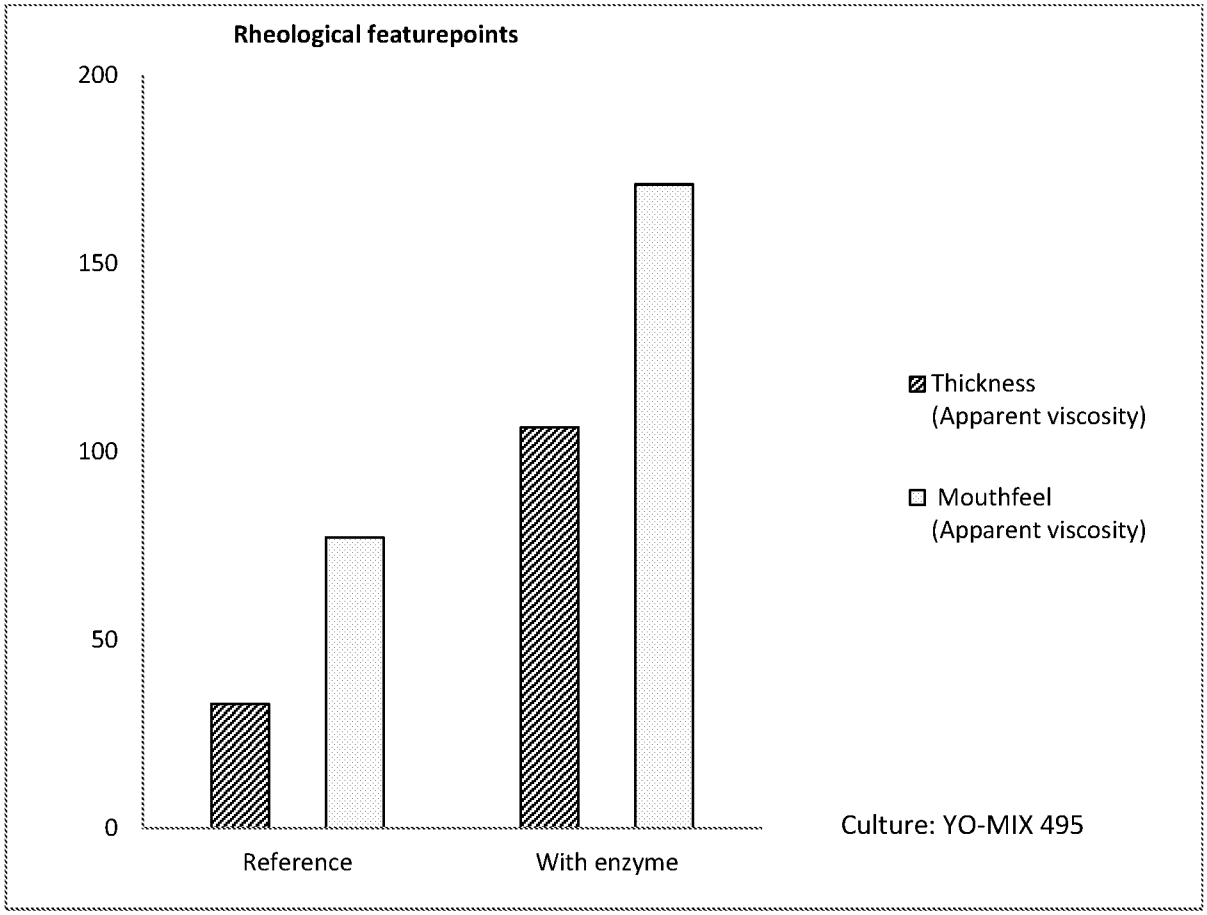


Fig. 2C - (YO-MIX 465) Thickness and mouthfeel as evaluated at 5 days post GTF300 treatment

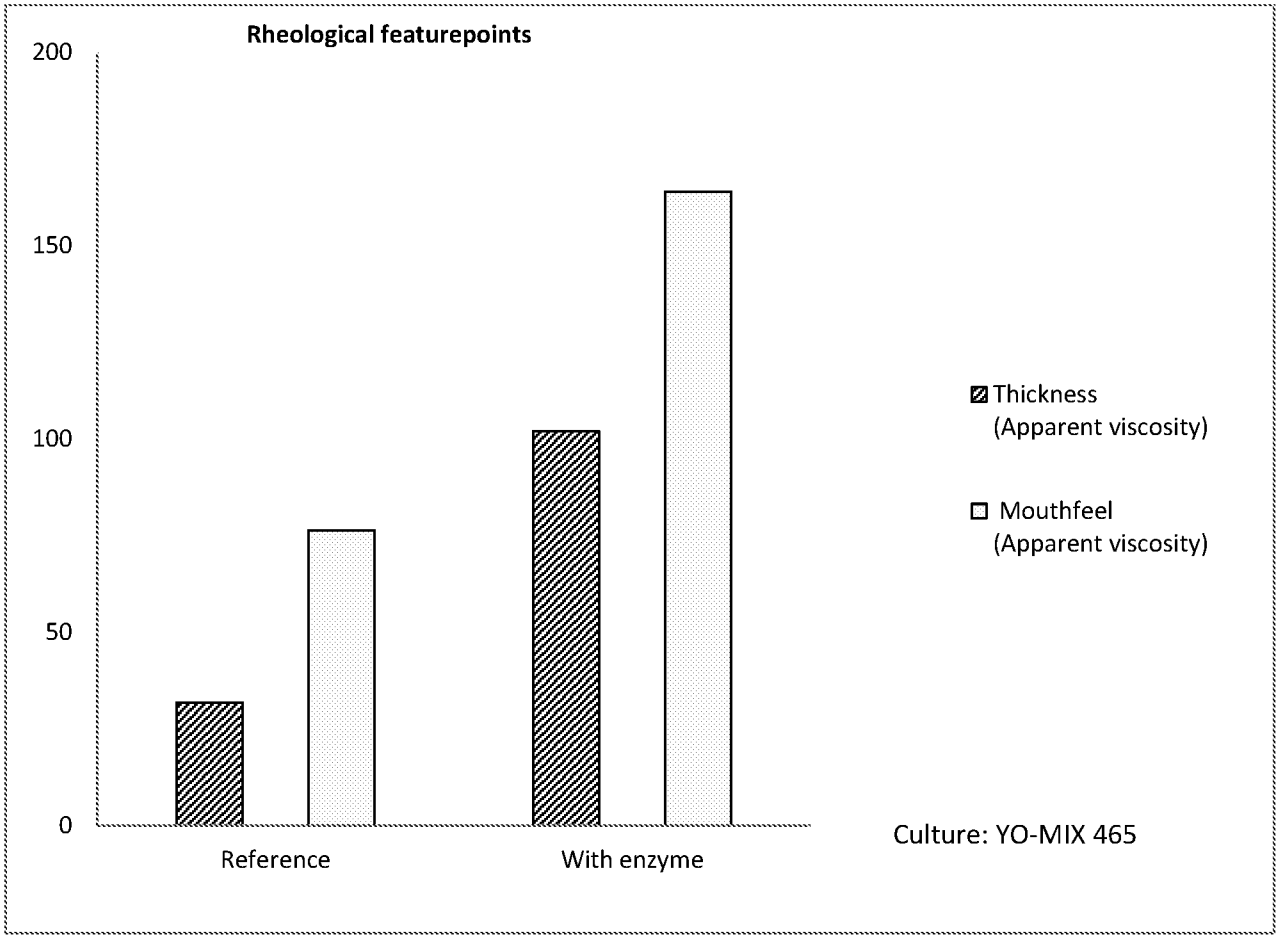


Fig. 3A - (YO-MIX 860) Thickness and mouthfeel as evaluated at 28 days post GTF300 treatment

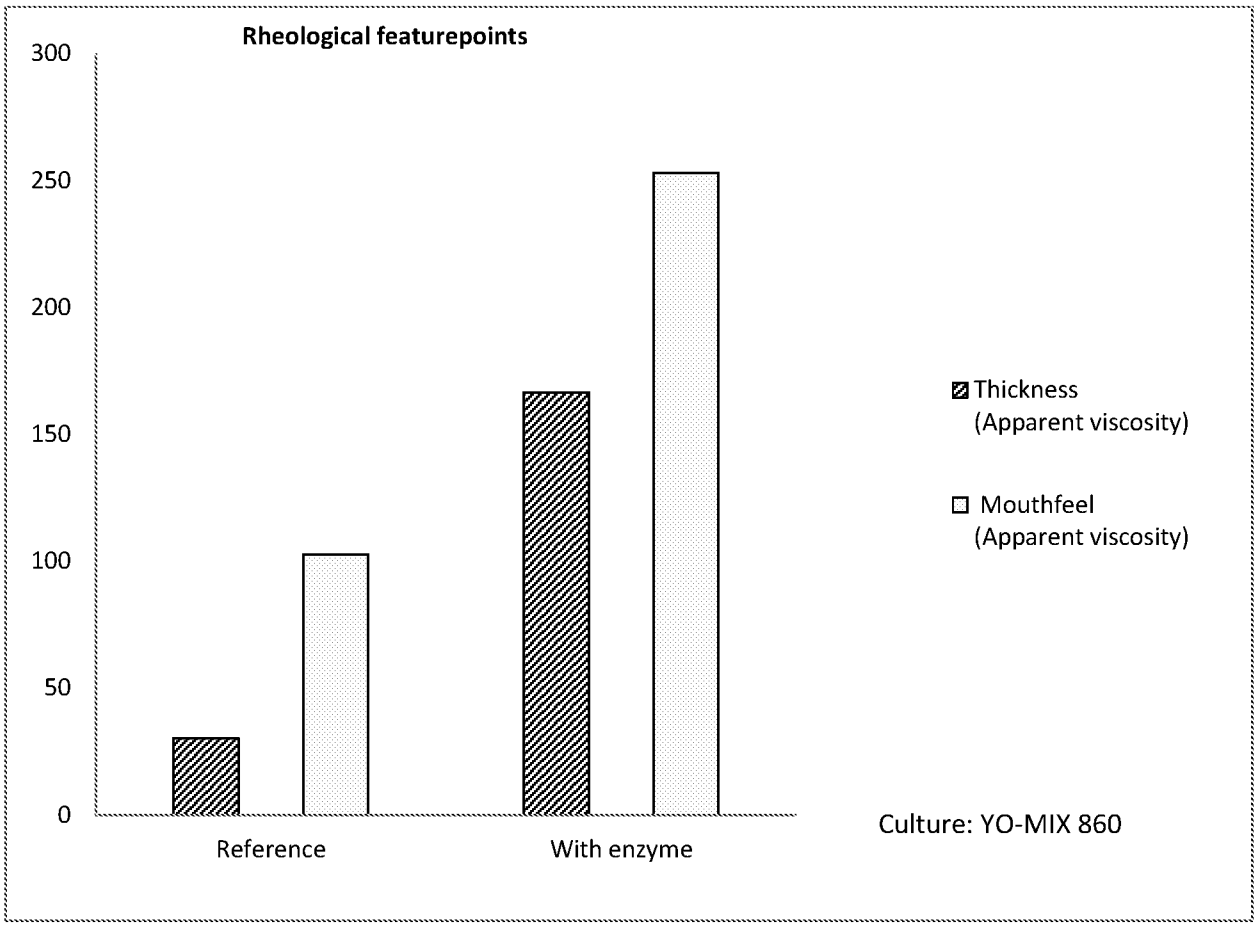


Fig. 3B - (YO-MIX 495) Thickness and mouthfeel as evaluated at 28 days post GTF300 treatment

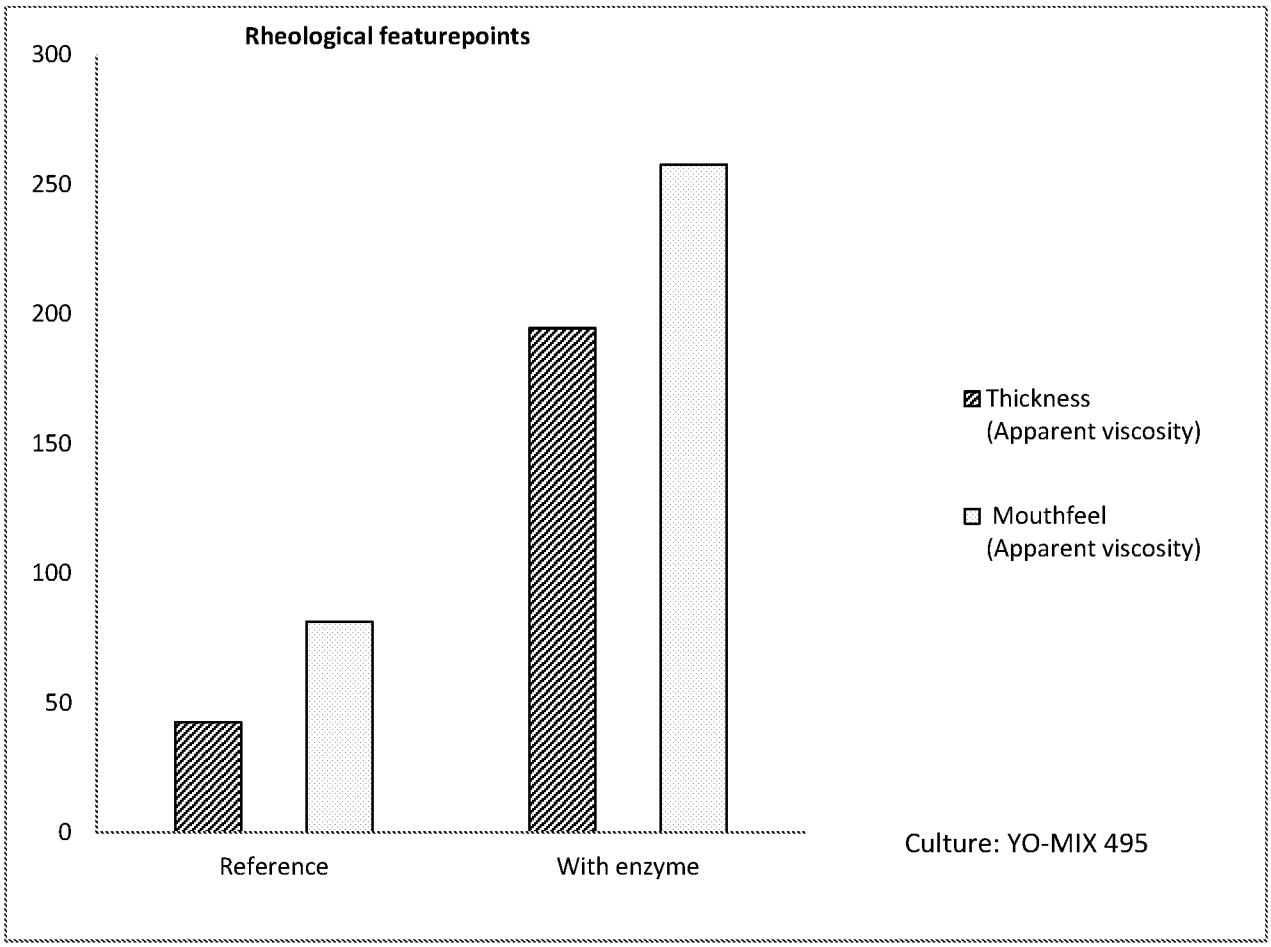


Fig. 3C - (YO-MIX 465) Thickness and mouthfeel as evaluated at 28 days post GTF300 treatment

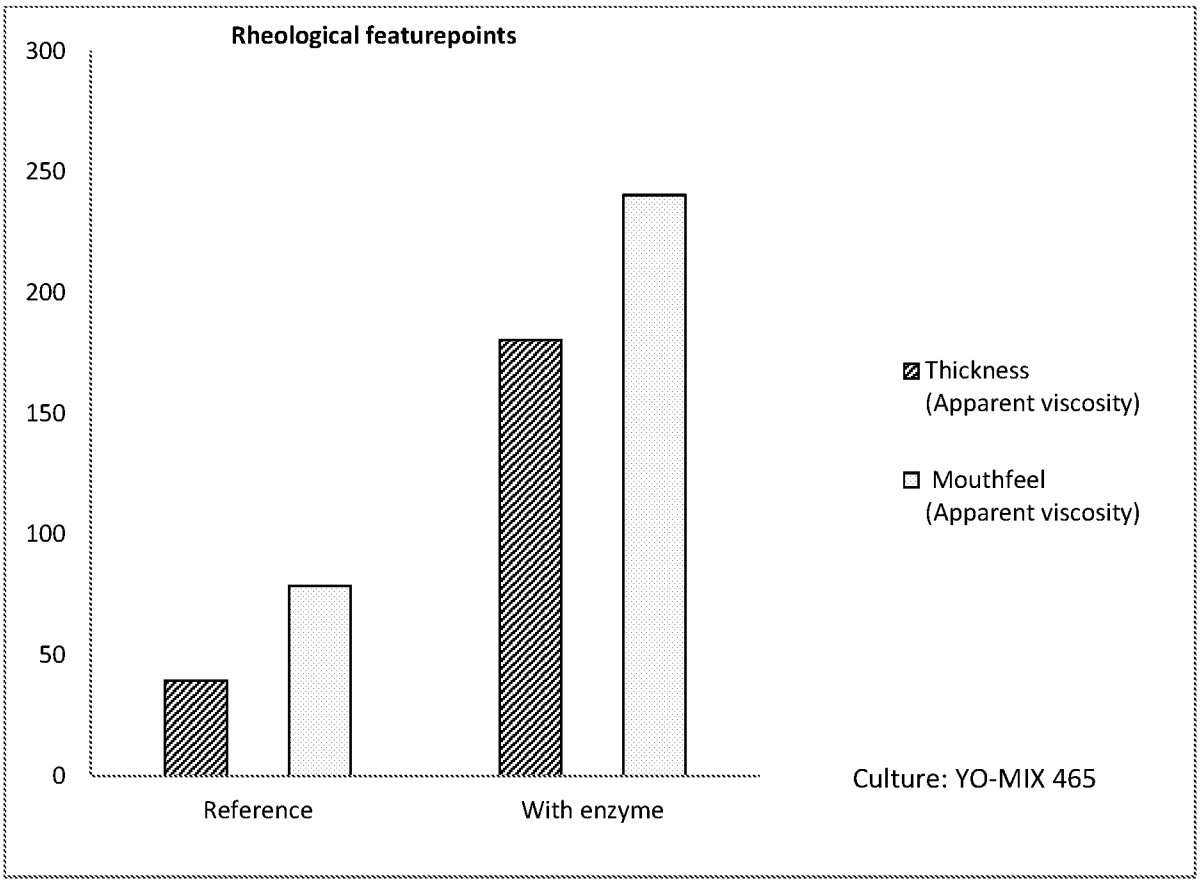


Fig. 4 Flow Diagram for Addition of Enzyme Prior to Pasteurization and Homogenization

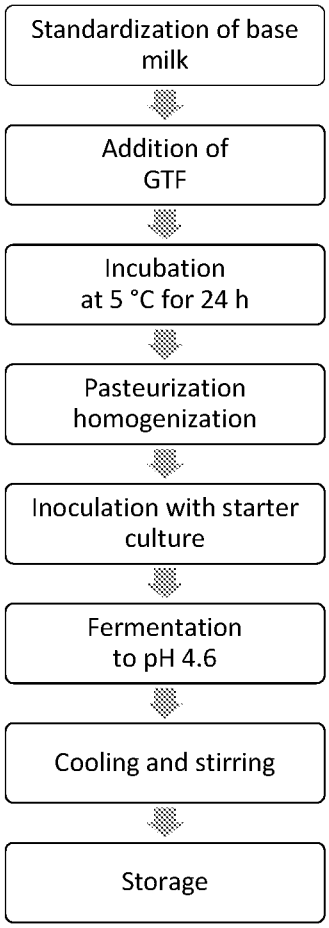


Fig. 5A - (YO-MIX 495) thickness and mouthfeel as evaluated at 7 days post GTF300 treatment with addition of enzyme before pasteurization and homogenization

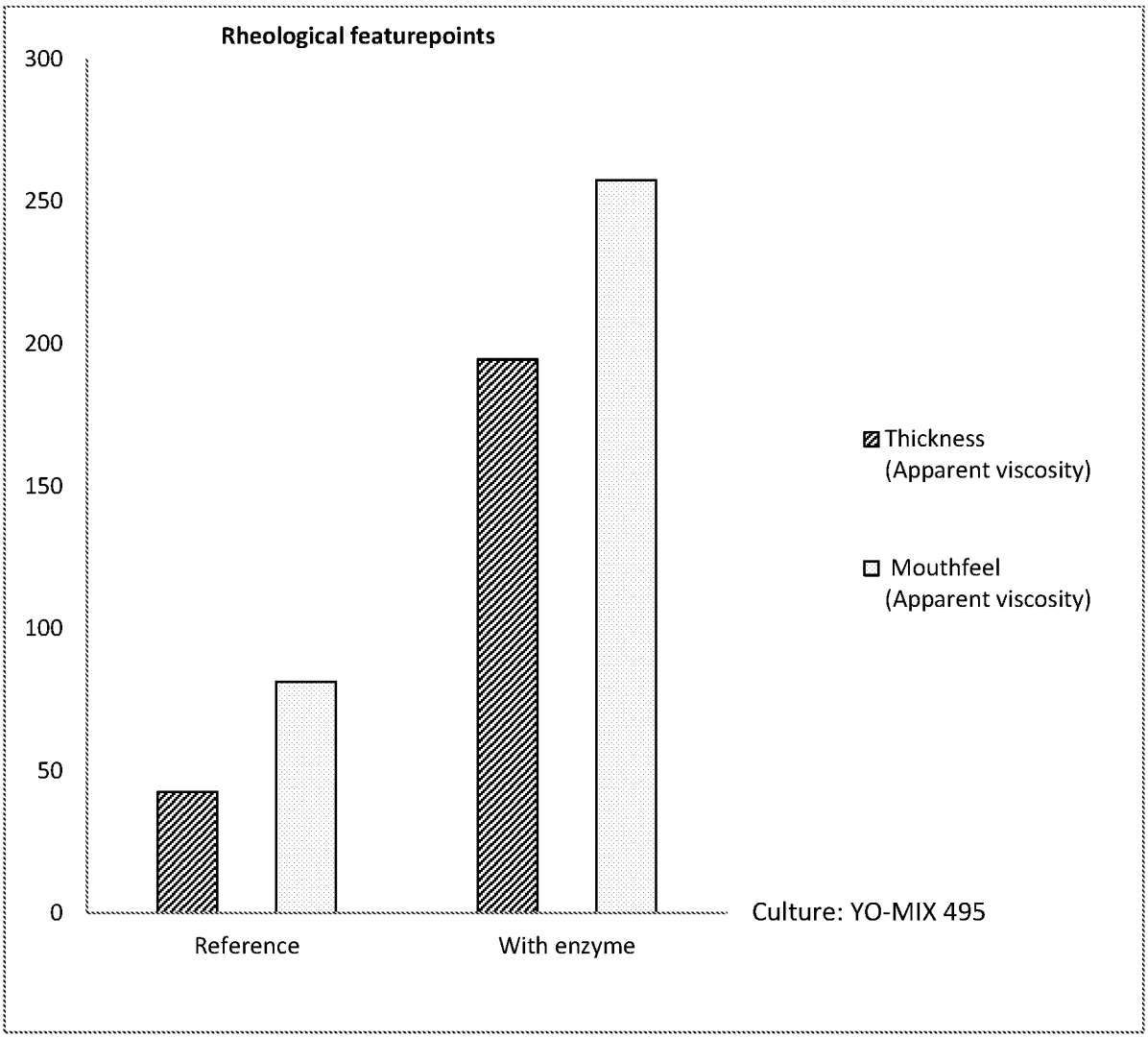


Fig. 5B - (YO-MIX 465) thickness and mouthfeel as evaluated at 7 days post GTF300 treatment with addition of enzyme before pasteurization and homogenization

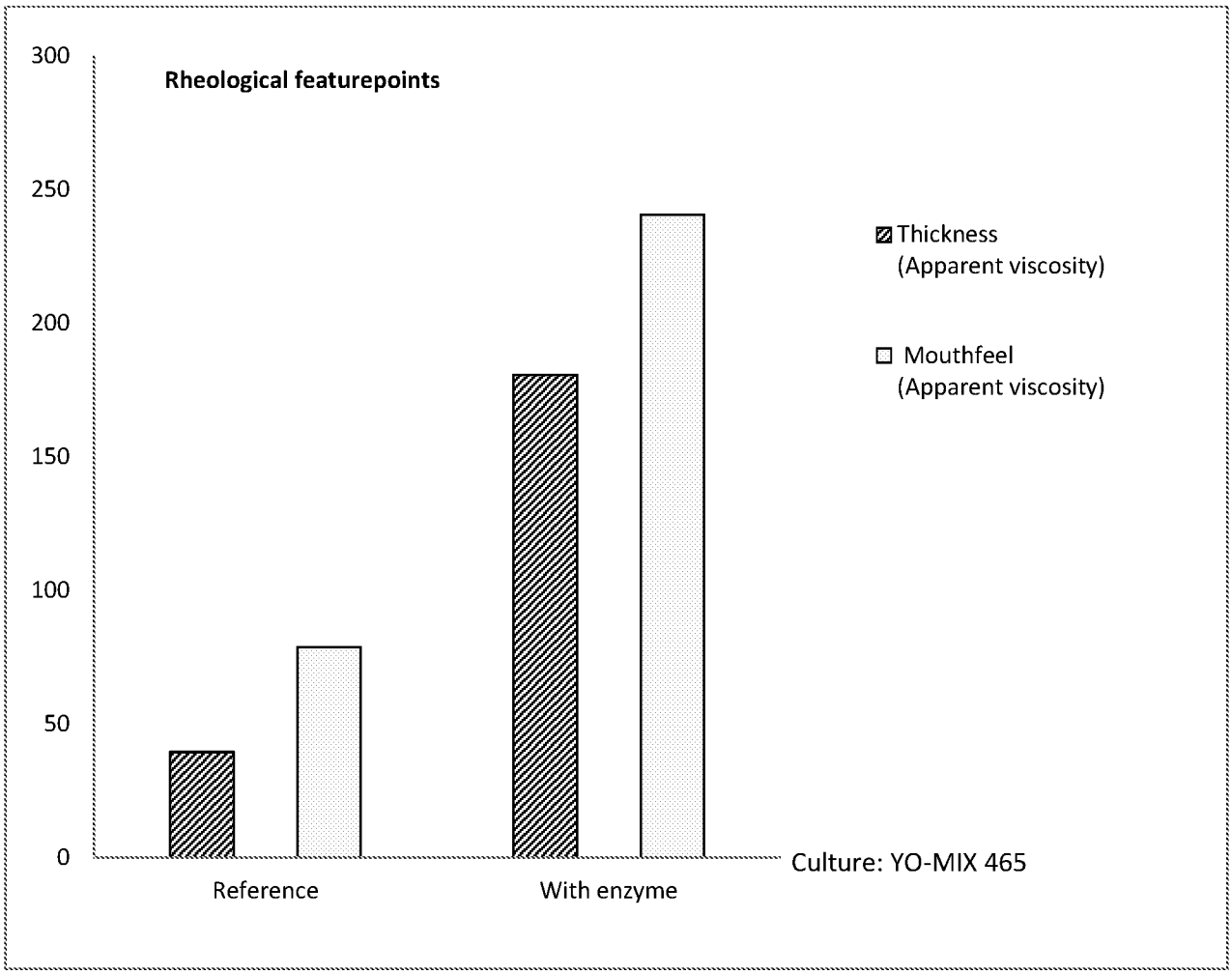


Fig. 5C - (YO-MIX 860) thickness and mouthfeel as evaluated at 7 days post GTF300 treatment with addition of enzyme before pasteurization and homogenization

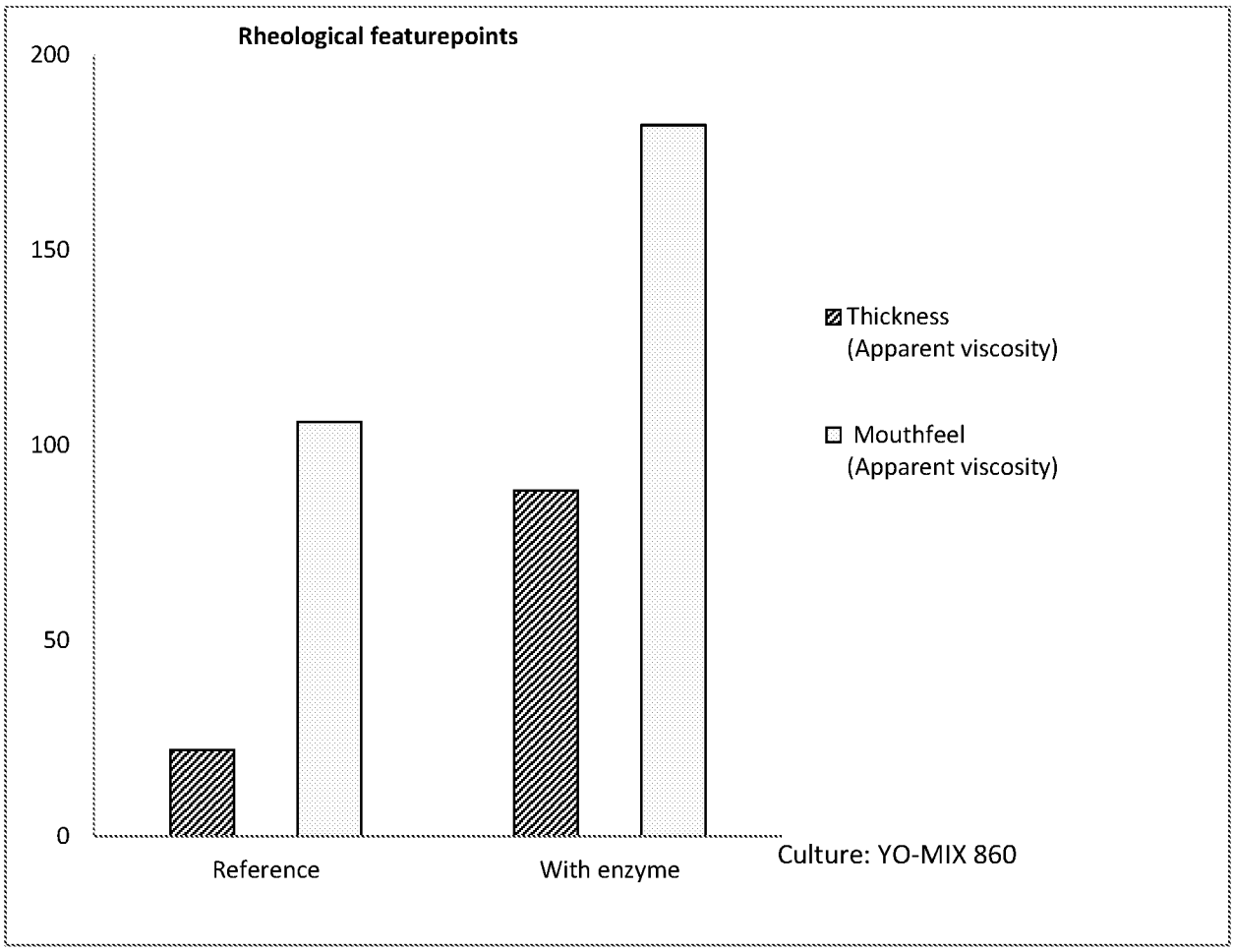


Fig. 5D - (YO-MIX 204) thickness and mouthfeel as evaluated at 7 days post GTF300 treatment with addition of enzyme before pasteurization and homogenization

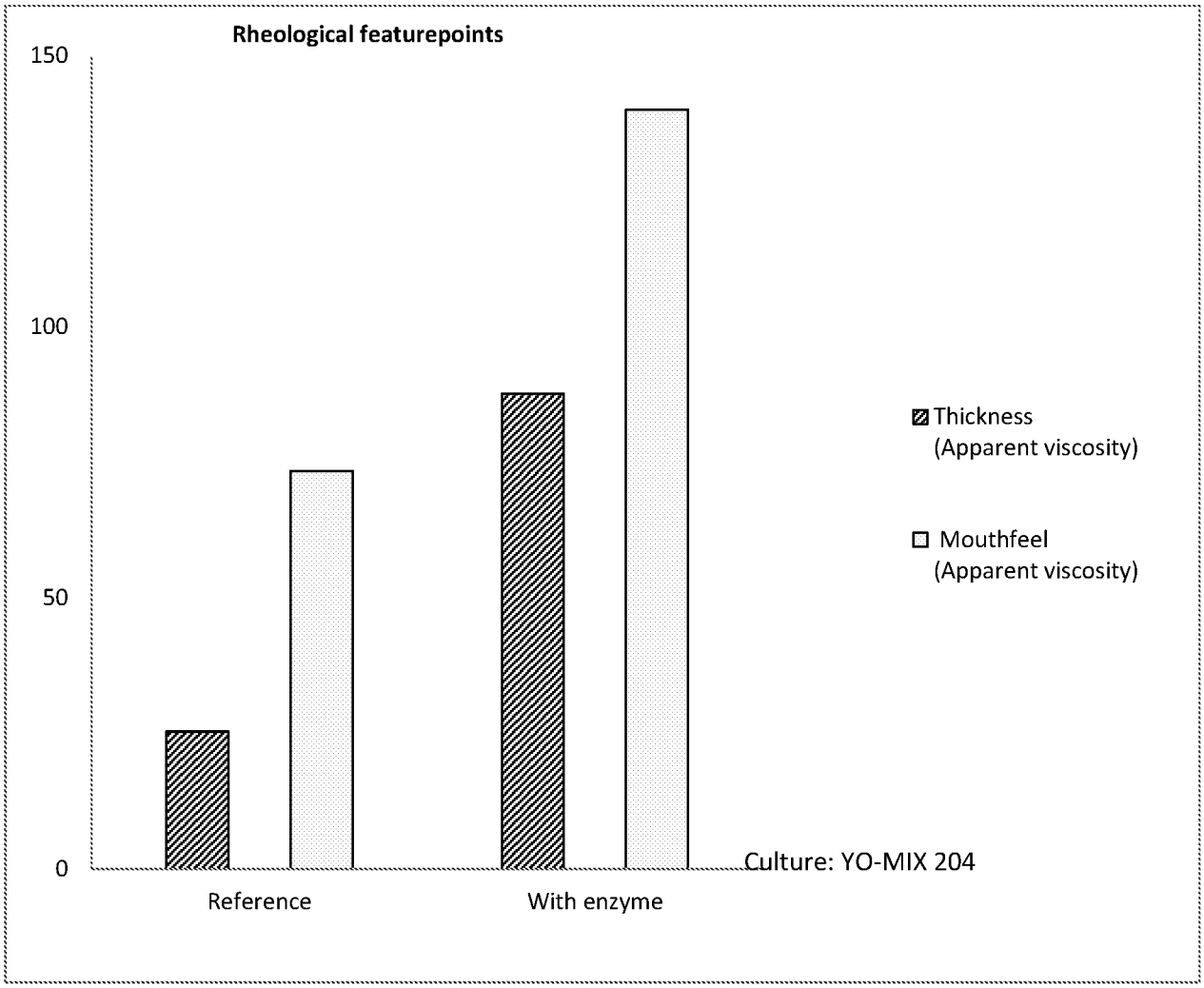


Fig. 6A - (YO-MIX 860) thickness and mouthfeel as evaluated at 28 days post GTF300 treatment with addition of enzyme before pasteurization and homogenization

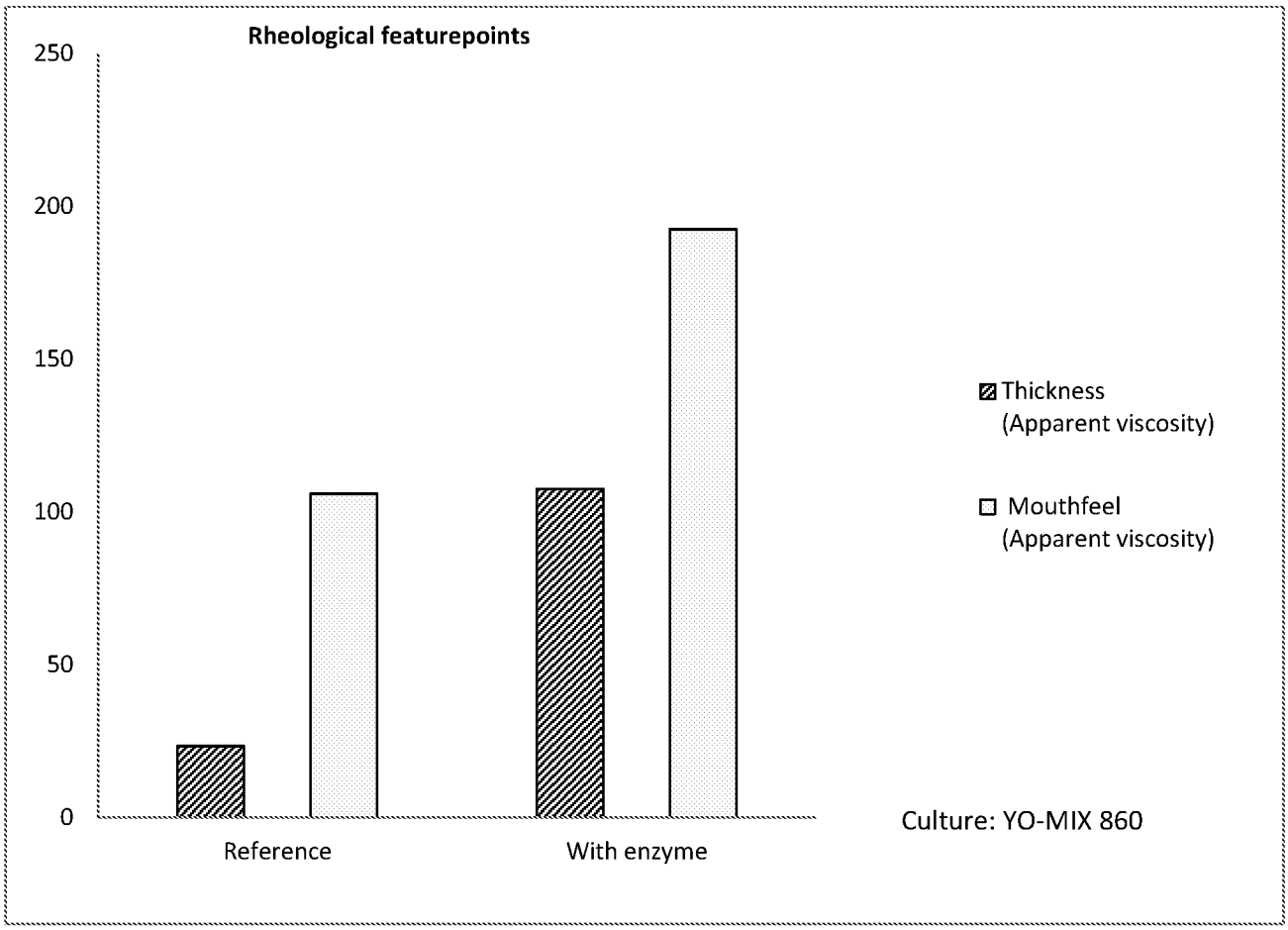


Fig. 6B - (YO-MIX 495) thickness and mouthfeel as evaluated at 28 days post GTF300 treatment with addition of enzyme before pasteurization and homogenization

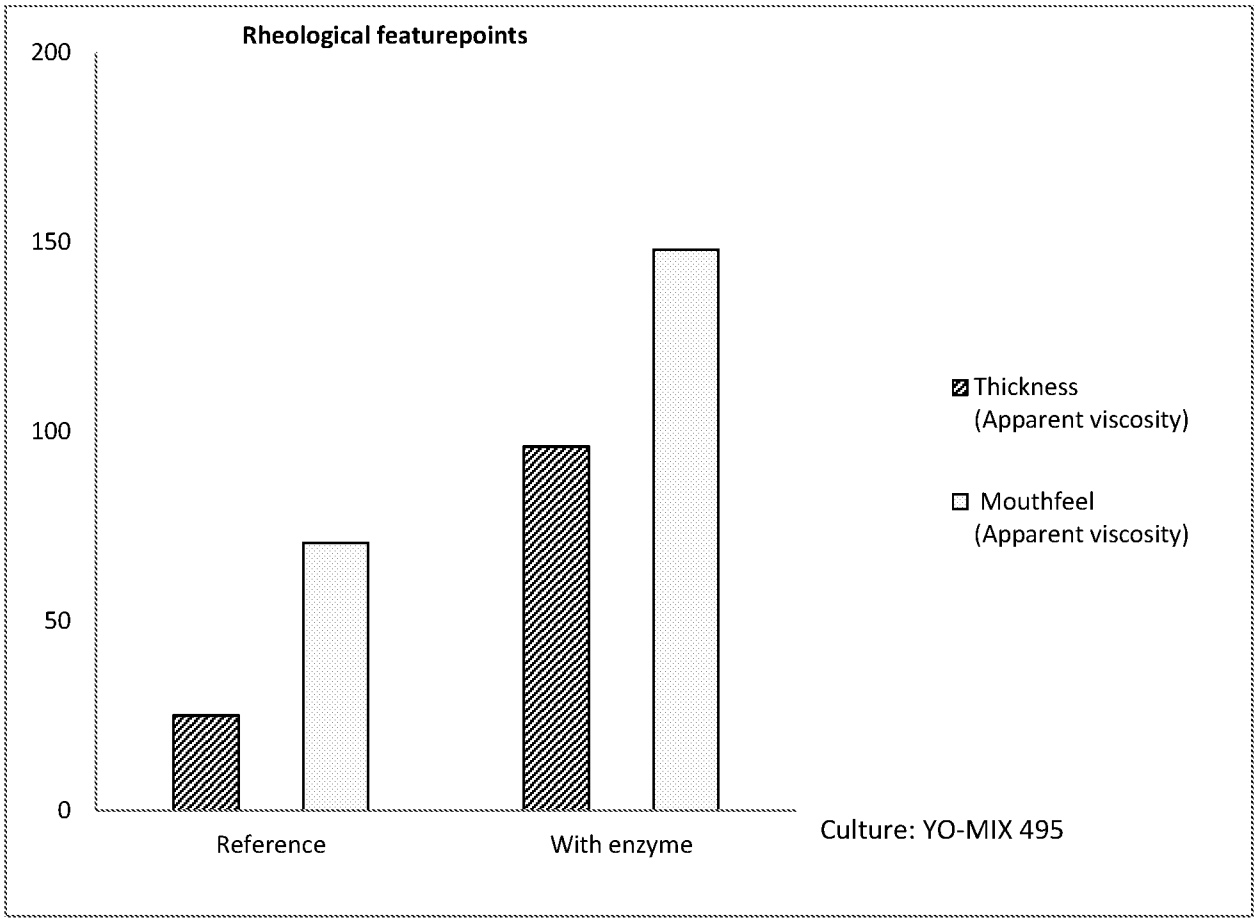


Fig. 6C - (YO-MIX 465) thickness and mouthfeel as evaluated at 28 days post GTF300 treatment with addition of enzyme before pasteurization and homogenization

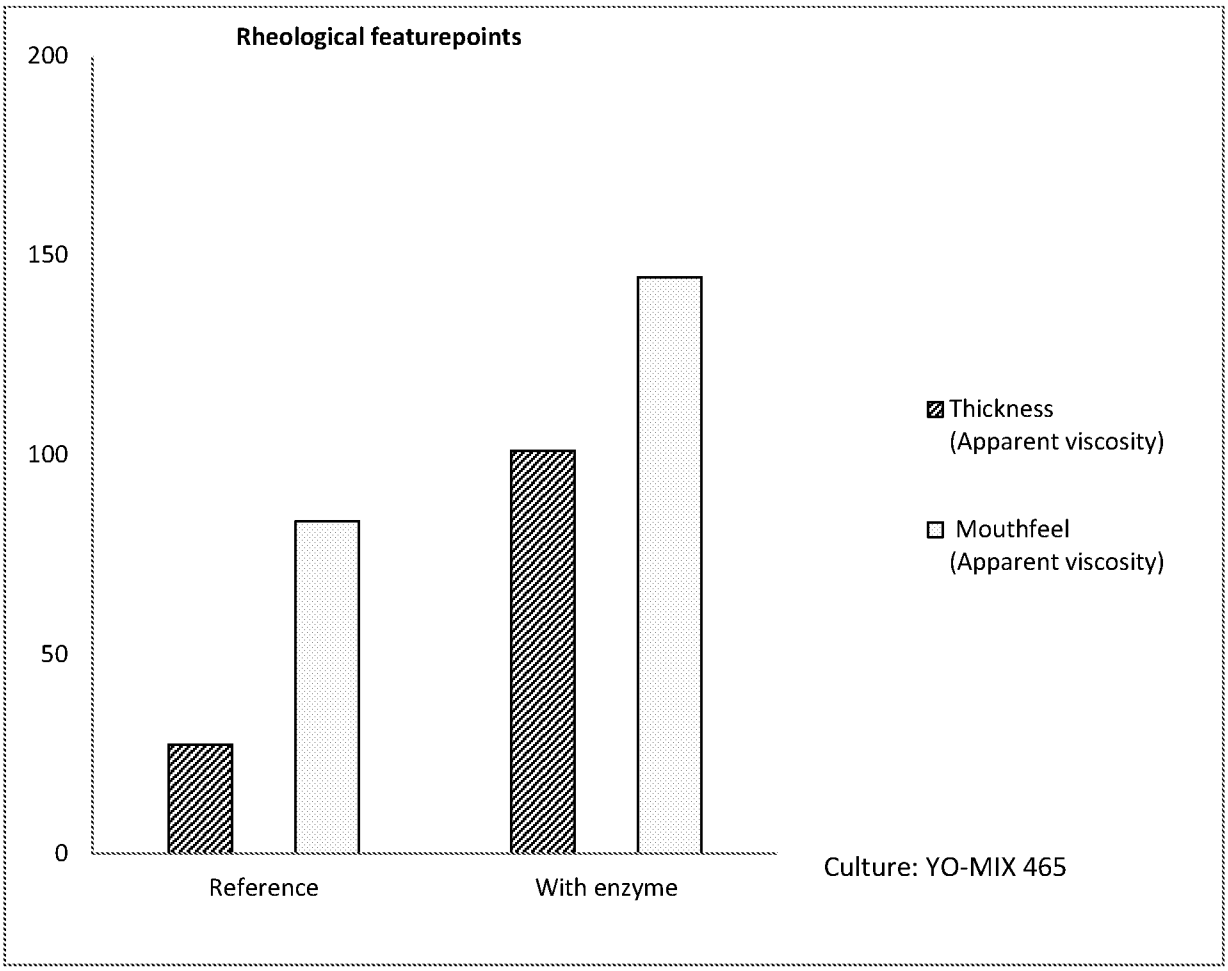


Fig. 6D - (YO-MIX 204) thickness and mouthfeel as evaluated at 28 days post GTF300 treatment with addition of enzyme before pasteurization and homogenization

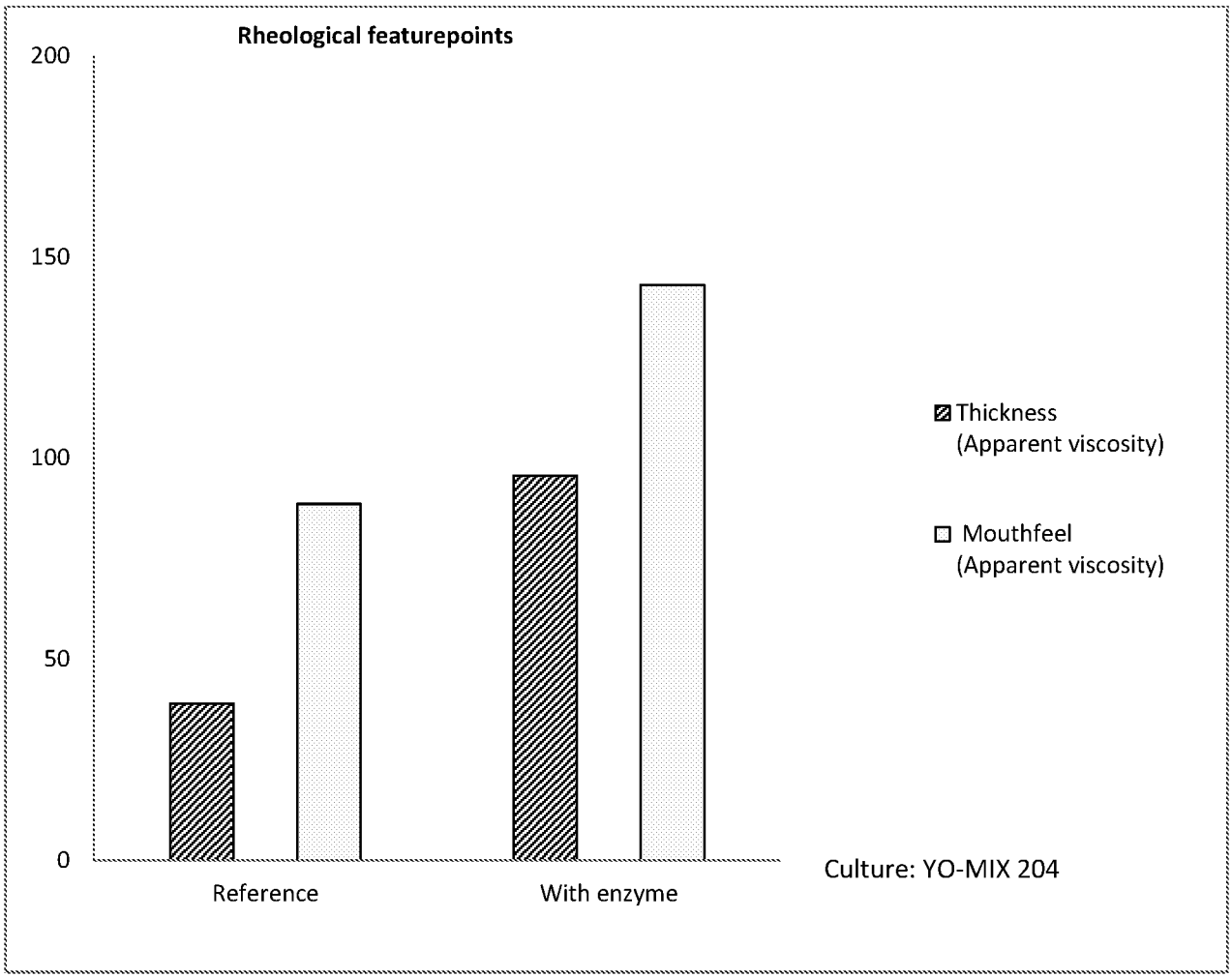


Fig. 7A - (2% sucrose) thickness and mouthfeel as evaluated with GTF300 addition before pasteurization and homogenization

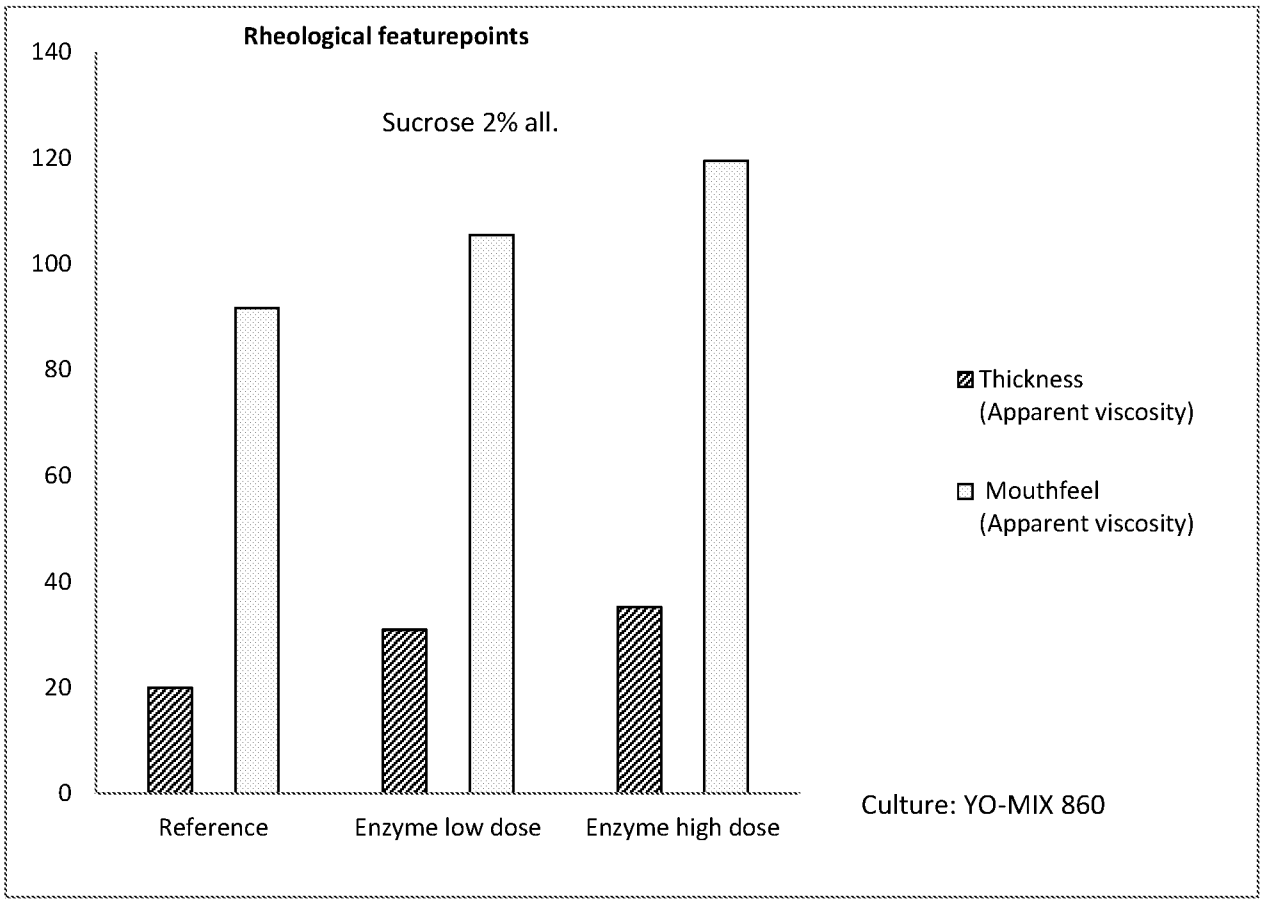


Fig. 7B - (4% sucrose) thickness and mouthfeel as evaluated with GTF300 addition before pasteurization and homogenization

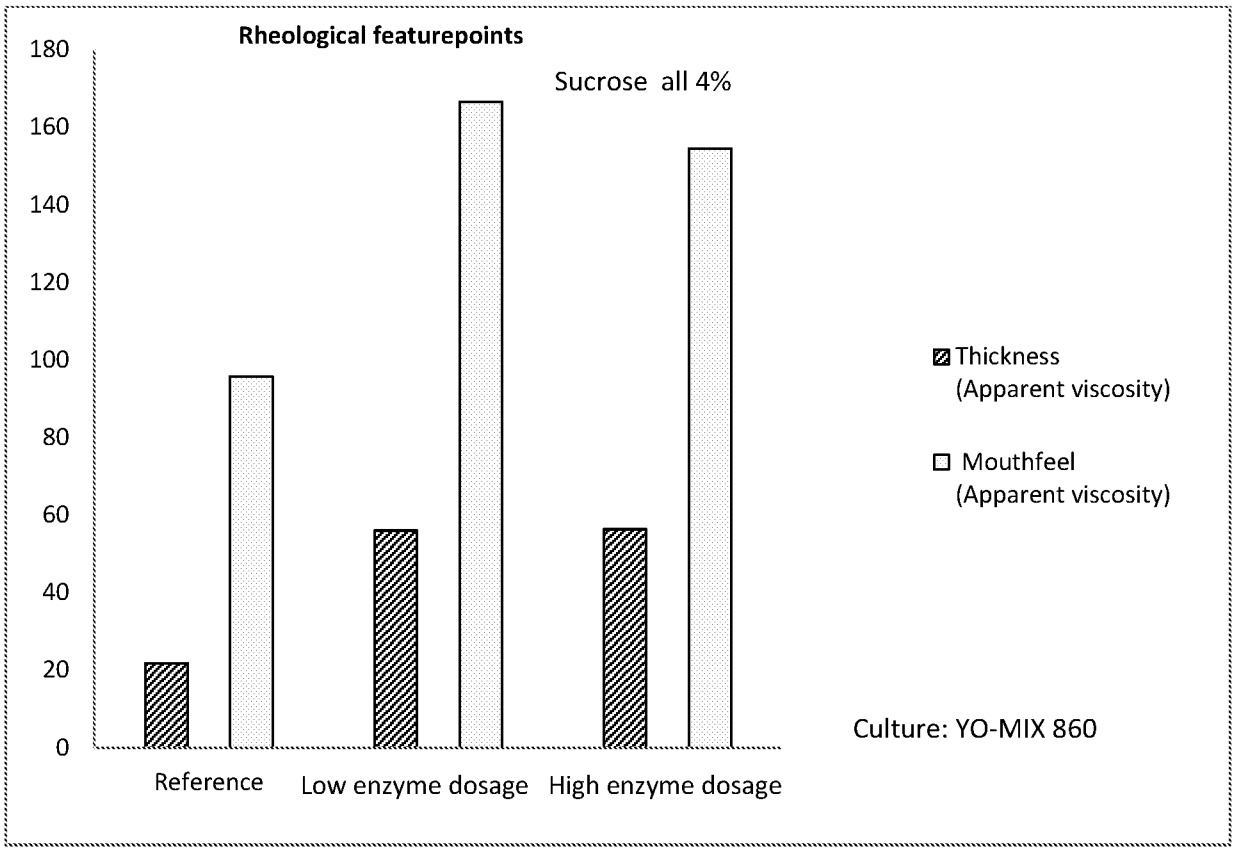


Fig. 8 - Effect of Yogurt Cooling to Different Temperatures on Texture and Mouthfeel after Filling (enzyme = GTF300)

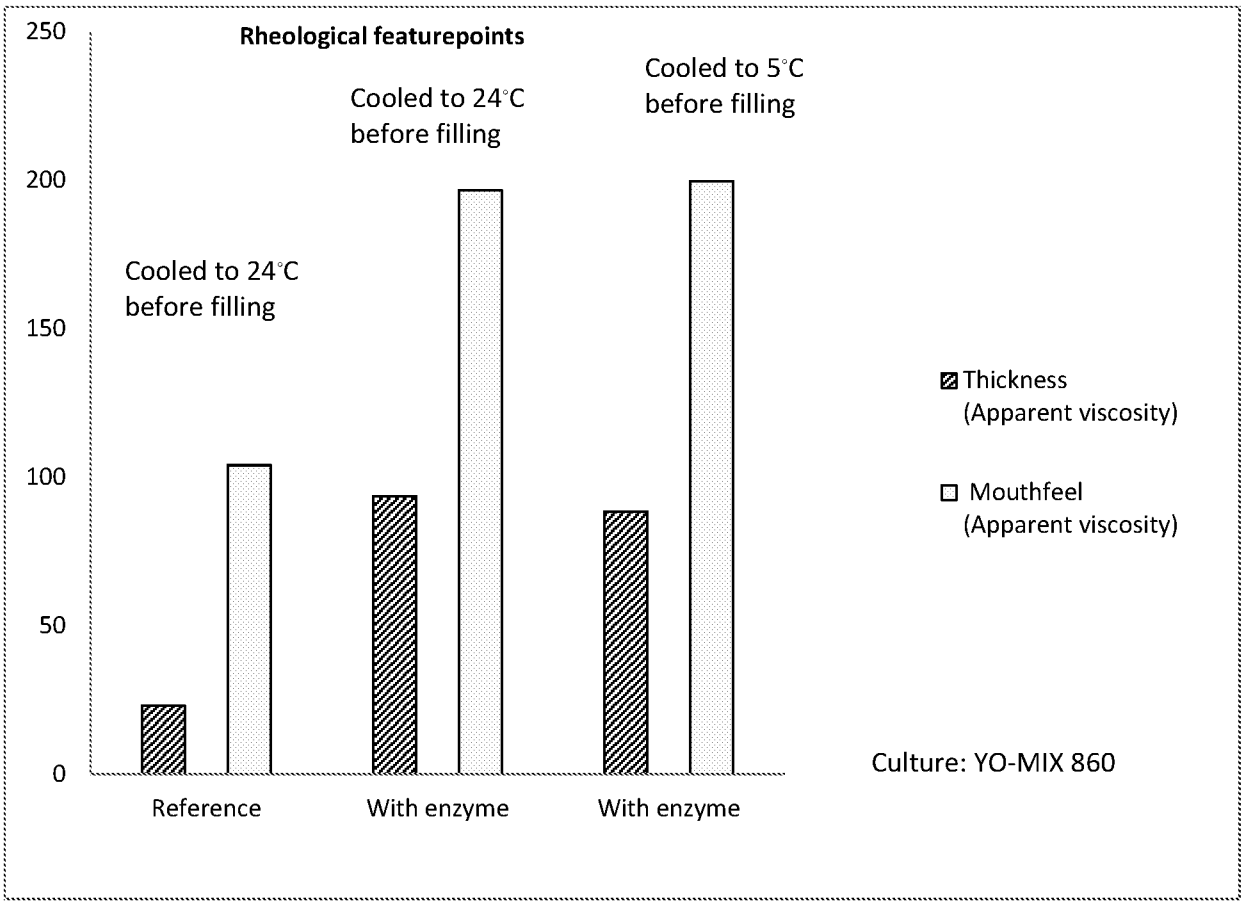


Fig. 9A - shows effect of GTFJ as function of enzyme concentration

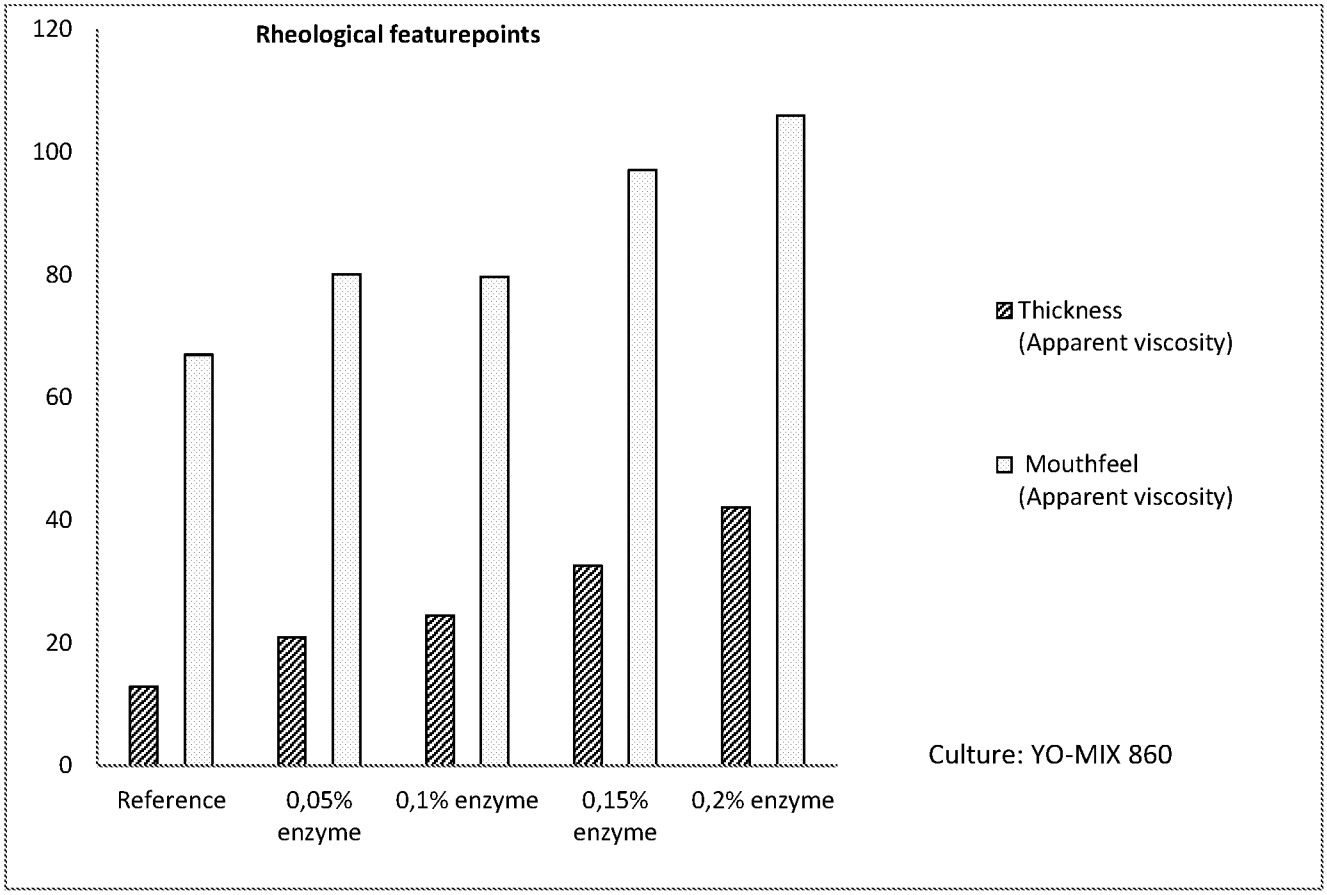
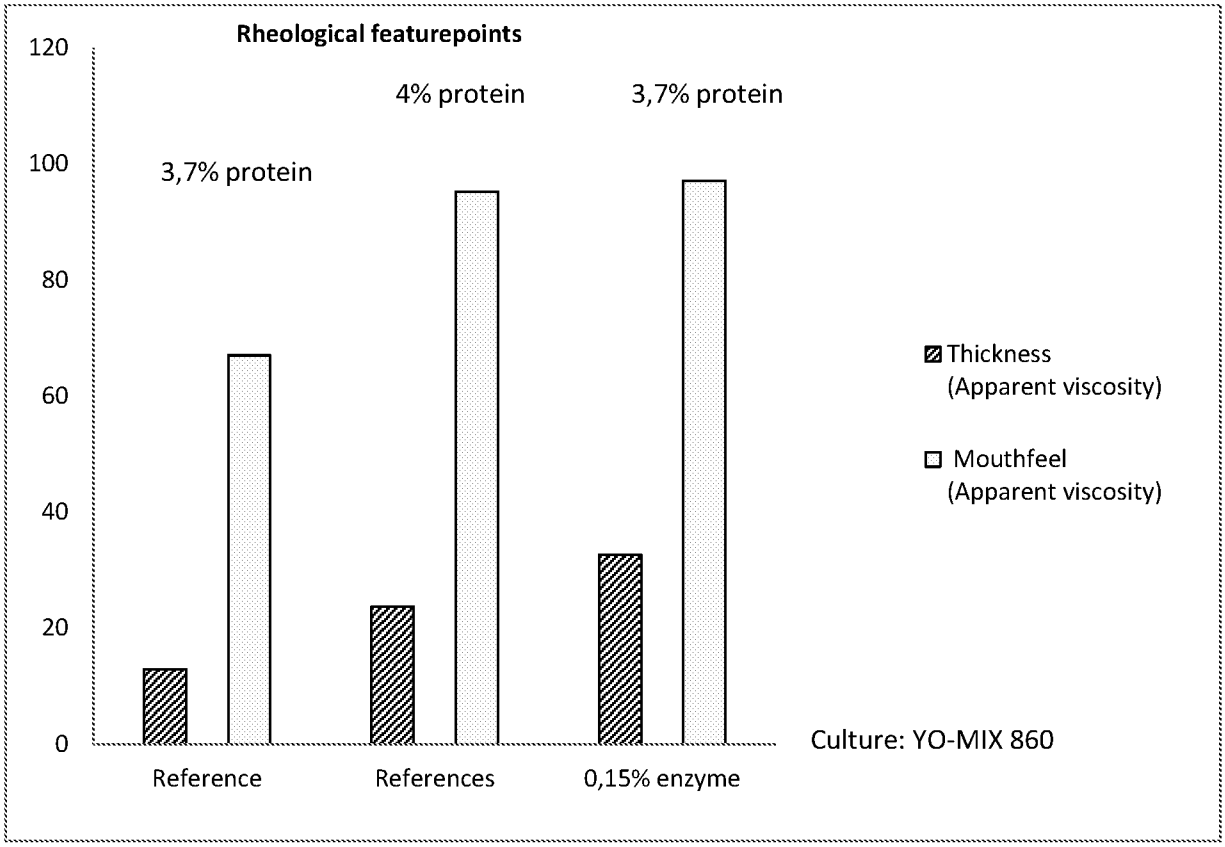


Fig. 9B - shows the effect of GTFJ as compared with a 4% protein yogurt



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/39447

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

An invitation to Furnish Nucleotide and/or Amino Acid Sequence Listing and to Pay, Where Applicable, Late Furnishing Fee ("ISA/225") was mailed on 15 July 2019 (15.07.2019). No approved electronic sequence listing was received in response to the ISA/225. Therefore, the international search was carried out only to the extent possible

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/39447

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 8-50
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/39447

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A23C 9/00, 9/12, 9/123, 9/127 (2019.01)

CPC - A23C 9/00, 9/12, 9/123, 9/127, 9/1234, 9/1238, 9/1275; C12N 1/20, 9/1048; C12P 19/04; C12R 1/46, 1/225

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0348980 A1 (CHR. HANSEN A/S) 27 November 2014; paragraphs [0009], [0012], [0030], [0031], [0038], [0045]	1-4, 5/1-4, 6/5/1-4, 7/5/1-4
A	US 2012/0107450 A1 (FOLKENBERG et al.) 03 May 2012; entire document	1-4, 5/1-4, 6/5/1-4, 7/5/1-4
A	US 8,309,076 B2 (KANG et al.) 13 November 2012; entire document	1-4, 5/1-4, 6/5/1-4, 7/5/1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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