Title: METHOD OF PREPARING AN EGG COMPOSITION

Abstract: The present invention relates to a method for preparing an egg comprising composition. More in specific, the invention relates to a method for stabilizing such a composition. The present invention provides a method for improving the stability of an egg, fat and sugar composition comprising adding a phospholipase to said composition.
METHOD OF PREPARING AN EGG COMPOSITION

Field of the invention

The present invention relates to a method for preparing an egg comprising composition. Specifically, the invention relates to a method for stabilizing such a composition.

Background of the invention

Baked products are prepared from dough which is usually made from the basic ingredients flour, water and optionally salt. Depending on the baked products, other optional ingredients are sugars, flavours etceteras. For leavened products, primarily baker's yeast is used next to chemical leavening systems such as a combination of an acid (generating compound) and bicarbonate. In order to improve the handling properties of the dough and/or the final properties of the baked products there is a continuous effort to develop processing aids with improving properties. Dough properties that are to be improved comprise machineability, gas retaining capability, etcetera. Properties of the baked products that may be improved comprise (loaf) volume, crust crispiness, crumb texture and softness, taste and flavour and shelf life. The currently existing processing aids can be divided into two groups: chemical additives and enzymes.

Chemical additives with improving properties comprise oxidising agents such as ascorbic acid, bromate and azodicarbonate, reducing agents such as L-cysteine and glutathione, emulsifiers acting as dough conditioners such as diacetyl tartaric esters of mono/diglycerides (DATEM), sodium stearoyl lactylate (SSL) or calcium stearoyl lactylate (CSL), or acting as crumb softeners such as glycerol monostearate (GMS) etceteras, fatty materials such as triglycerides (fat) or lecithin and others.

Presently, there is a trend to replace the chemical additives by enzymes. The latter are considered to be more natural compounds and therefore more accepted by the consumer. Suitable enzymes may be selected from the group consisting of starch degrading enzymes, arabinoxylan- and other hemicellulose degrading enzymes,
cellulose degrading enzymes, oxidizing enzymes, fatty material splitting enzymes and protein degrading enzymes.

One non-limiting example of a baked product is a cake. This example will be explained, without being restricted to it, in more detail.

Cake is known for a long time and is prepared in numerous varieties. Most cakes are made with wheat flour and therefore have some amount of gluten, which means special care needs to be taken to ensure cakes don't have a chewy texture. The cake ingredients are mixed as little as possible once the flour has been added. This differs markedly from sturdy food items made with flour such as bread, where the goal is to agitate the gluten as much as possible. The wheat flour selected to be used for cakes is often one naturally lower in gluten.

Typical cake ingredients are wheat flour, eggs and sugar. Optionally, baking powder, water, and/or fat - such as for example butter, margarine and/or oil are added.

Cakes often rely on beating eggs and addition of leavening agents, such as baking powder, to produce the air bubbles in the cake. This is what makes a traditional cake fluffy and sponge-like. Therefore the type of cake ingredients and the ratio between them are important in determining cake properties such as e.g. crumb structure and cake volume.

Whipping agents (emulsifiers), such as polyglycerol esters, monoglycerides or others, are also used in cake baking.

In cake recipe's eggs are used as providers of natural emulsifiers mainly due to the presence of phospholipids that have surface-active properties. Whole eggs contain 11% lipids of which 25% is lecithin and they contain about 13% of protein.

The fat is added to entrap air during mixing, for lubrication to improve the overall eating quality in terms of moisture and tenderness, to improve the structure of the finished product, and/or to extend shelf life.

To decrease the amount of steps needed by a manufacturer of a baked product (for example a cake manufacturer) and as a result to decrease the chance of a mistake, it is desired that more ingredients or as many ingredients as possible are already added together before arriving at the cake manufacturer. However, the adding together of multiple ingredients may not negatively affect the features of a final baked product. It is
therefore an object of the present invention to premix multiple ingredients used in the preparation of a baked product essentially without negatively affecting the final features of a baked product. Moreover, it is an object of the present invention that the composition of the different ingredients is present as a homogeneous composition such that it can be used directly by a manufacturer, i.e. the prepared composition of ingredients must be stable, i.e. must not segregate into different phases. It is a further object of the present invention to improve desired baked product (for example cake) properties such as e.g. crumb structure and/or volume in a baked product.

The objective of the present invention is reached by the use of a phospholipase during the combining of certain ingredients.

**Description of the Figures**

Figure 1: Viscosity of (right side) sugared whole egg dispersion versus (left side) the viscosity of the sugared whole egg liquid butter.

**Summary of the invention**

The inventors of the present invention have surprisingly noted that an egg comprising composition can be improved by adding a phospholipase. The herein described methods result in a composition or blend (the terms are used interchangeably herein) with, for example, improved stability.

A composition (or blend) produced as described herein performs better in a baking application when compared to the situation in which the separate ingredients of the composition were added as such.

Another surprise was that a composition as obtained or described herein has improved microbial spoilage features, i.e. the composition is less susceptible to microbial growth.

**Detailed description of the invention**

The present invention provides a method for improving the stability of an egg, fat and sugar composition comprising adding a phospholipase to said composition. Alternatively worded, the invention provides a method in which a composition comprising egg, fat and sugar is stabilized by adding a phospholipase to said composition, i.e. no - by the eye- visible segregation occurs. As will be explained in
more detail later on, the phospholipase is allowed to incubate for a certain amount of
time and at an appropriate temperature to obtain an improved stability of said
composition. Such a method typically further comprises a mixing step in which the
components are mixed.

The term "stability" as used herein refers to a situation in which the composition
does, at least visibly, not or hardly not segregate into different layers. Preferably, the
stability (or homogenicity) of said composition is acceptable even after a period of
storage such as multiple days or even weeks. Storage of said composition can be at for
example room temperature or refrigerator temperatures (for example between 2 to 7
degrees Celsius). The phrase "improving the stability" is used to describe that the
composition treated with a phospholipase segregates less or at least later in time
compared to the same composition which is not treated with a phospholipase.

Alternatively worded, the invention provides a method for at least in part
decreasing or preventing segregation of an egg, sugar and fat comprising composition
wherein said method comprises adding a phospholipase to said composition.

The term "composition" as used herein typically refers to a composition suitable
for use in preparing a baked product, i.e. a baking composition. Such a composition
comprises at least egg, fat and sugar. Depending on the desired baked product such a
composition can comprise additional components such as baking powder, salt, water,
flavors, milk components, (modified) starch, hydrocolloids, fibers, enzymes (such as but
not limited to protease, oxidase, amylase and/or lipase), gum and/or an emulsifier.
Another optional component (or ingredient; the terms are used interchangeably herein)
of said composition is flour. In a preferred embodiment, the composition of the invention
is a so-called intermediate or premix baking composition, i.e. a composition which can
not directly be used in baking but to which composition at least one, preferably at least
two, additional ingredient(s) must be added before baking can take place. Examples of
such ingredients are flour, salt, egg white, baking powder or emulsifier. Alternatively
worded, the invention provides a method for improving the stability of an egg, fat and
sugar composition comprising adding a phospholipase to said composition, with the
proviso that said composition is not a ready for use baking composition (i.e. is not a
batter) or more generally phrases, with the proviso that said composition is not ready
for preparing a food stuff. In another alternative embodiment, the invention provides a
method for improving the stability of an egg, fat and sugar composition comprising
adding a phospholipase to said composition, wherein said composition does not comprise flour.

In yet another alternative embodiment, the invention provides a method for improving the stability of an egg, fat and sugar consisting composition (i.e. a composition consisting of egg, fat and sugar) comprising adding a phospholipase to said composition.

The term “egg” as used herein refers to a whole egg (for the avoidance of doubt: without its eggshell), egg white or to egg yolk. Preferably, the invention provides a method for improving the stability of an egg, fat and sugar composition comprising adding a phospholipase to said composition, wherein said egg is whole egg or egg yolk. As outlined in more detail below, said egg (optionally in combination with sugar) is treated with phospholipase before being mixed with said sugar and fat (optionally before being mixed with fat) to obtain phospholipase treated egg or phospholipase treated egg-sugar. In a preferred embodiment, the phospholipase treated egg or phospholipase treated egg-sugar is not dried but is kept as a non-dried (liquid or partly solidified) composition.

The term “fat” as used herein refers to a fat which is typically used in the preparation of a certain baked product. The skilled person is capable of selecting the right kind of fat in respect of a certain baked product.

Fats consist of a wide group of compounds that are generally soluble in organic solvents and largely insoluble in water. Chemically, fats are generally triesters of glycerol and fatty acids. Fats may be either solid or liquid at normal room temperature, depending on their structure and composition. Although the words "oils", "fats", and "lipids" are all used to refer to fats, "oils" is usually used to refer to fats that are liquids at normal room temperature, while "fats" is usually used to refer to fats that are solids at normal room temperature. "Lipids" is used to refer to both liquid and solid fats, along with other related substances. The word "oil" is used for any substance that does not mix with water and has a greasy feel, such as petroleum (or crude oil) and heating oil, regardless of its chemical structure.

Examples of edible animal fats are lard (pig fat), fish oil, butter or ghee. They are obtained from fats in the milk, meat and under the skin of the animal. Examples of edible plant fats are peanut, soy bean, sunflower, sesame, coconut, olive, and vegetable oils. Margarine and vegetable shortening can be derived from the above
oils. These examples of fats can be categorized into saturated fats and unsaturated fats.

Butter is a dairy product made by churning fresh or fermented cream or milk. It is generally used as a spread and a condiment, as well as in cooking applications such as baking, sauce making, and frying. Butter consists of butterfat, water and milk proteins. Most frequently made from cows' milk, butter can also be manufactured from that of other mammals, including sheep, goats, buffalo, and yaks. Salt, flavorings and preservatives are sometimes added to butter. Rendering butter produces clarified butter or ghee, which is almost entirely butterfat.

Butter is an emulsion which remains a solid when refrigerated, but softens to a spreadable consistency at room temperature, and typically melts to a thin liquid consistency at 32-35°C.

Commercial butter is about 80% butterfat and 15% water; traditionally-made butter may have as little as 65% fat and 30% water. Butterfat consists of many moderate-sized, saturated hydrocarbon chain fatty acids.

In a preferred embodiment, the used fat is butter. An example of a suitable butter is butter oil. The invention therefore provides a method for improving the stability of an egg, fat and sugar composition comprising adding a phospholipase to said composition, wherein said fat is butter, preferably butter oil. Butter oil is commercially available, for example from Friesland Foods. Another suitable fat is vegetable oil.

In yet another preferred embodiment, the fat is liquid and can be added directly to the egg or egg-sugar phospholipase treated composition. Preferably, the temperature of the phospholipase treated composition is above the crystallisation temperature of the used fat. Typically, the fat can be added to the phospholipase treated composition after said composition has been brought to a temperature of between 20-30 °C. A typical phospholipase treatment involves an incubation at a temperature which is higher than the mentioned 20-30 °C and in such a case the phospholipase treated composition is cooled down to the desired temperature (but above the crystallisation temperature of the used fat). Alternatively one can add the fat at the temperature which was used during the phospholipase treatment (for example 50 °C).

The term "sugar" as used herein typically refers to saccharose, glucose or carbohydrate syrups as C-Sweet F (hydrolsed maize from Cargill, a glucose - fructose
mix) or Frutasun O (hydrolyzed inuline from Suiker Unie a fructose - glucose mix) or any other sugar for incorporation into a baked product. In a preferred embodiment, the invention provides a method for improving the stability of an egg, fat and sugar composition comprising adding a phospholipase to said composition, with the proviso that said sugar is not maltodextrin.

The components of the above mentioned composition can be present in diverse amounts and ratios. In one the preferred embodiment, the components are present in a ratio of approximately 1:1:1 (egg: sugar: fat) based on weight percentage. Other suitable ratios are:

- egg: sugar: fat= 2:2:1, i.e. a composition comprising less fat; as will be explained in more detail later on a fat replacer such as Etenia can be used to compensate for the decreased amount of fat
- egg: sugar: fat= 2:1:2, i.e. a composition comprising less sugar; the taste can optionally be compensated by using an artificial sweetener
- egg: sugar: fat= 2:1:1, i.e. a composition comprising less sugar and less fat
- egg: sugar: fat= 1:2:2, i.e. a composition with relative decreased amount of egg
- egg: sugar: fat= 1:1:2, i.e. a composition with decreased amount of egg and sugar
- egg: sugar: fat= 1:2:1, i.e. a composition with decreased amount of egg and fat

In a preferred embodiment, the invention provides a method for improving the stability of an egg, fat and sugar composition comprising adding a phospholipase to said composition, wherein said egg is pre-incubated with said phospholipase (and subsequently added/mixed to/with sugar and fat) and even more preferably wherein said egg and said sugar is pre-incubated with said phospholipase (and subsequently added/mixed to/with fat). The time and temperature of a pre-incubation can vary depending on the phospholipase used. A(n) (preferred) incubation is performed at 4 to 55 degrees Celsius for 1 hour to 4 days. More preferred is an incubation at 45 to 55 degrees Celsius for 2 to 4 hours.

In yet another preferred embodiment, the invention provides a method for improving the stability of an egg, fat and sugar composition comprising adding a
phospholipase to said composition wherein said egg or said egg and said sugar are incubated with said phospholipase, adding fat and sugar to said phospholipase treated egg or adding fat to said phospholipase treated egg-sugar and wherein said method further comprises allowing the obtained egg, fat and sugar composition to stand at a temperature of 2-10°C for at least 24 hours. Alternatively worded, the invention further provides a method as described above, wherein the obtained composition is allowed to stand at a temperature of 2-10°C for at least 24 hours.

As will be shown in the experimental part, storing the obtained composition at around 4 °C further improved the stability of the obtained composition.

The term "for at least 24 hours" is used to refer to a storage period of for example 24 or 48 hours or for example to a storage period of 2-4 or 2-7 days. After incubation at 2-10°C for at least 24 hours, the composition can subsequently be stored at ambient temperatures (typically 18-22 °C). However, because of the fact that the composition comprises eggs it is preferred, from a hygienic point of view, to keep the prepared composition at 2-10°C. At such temperatures the stability (i.e no visible segregation) is very good.

As will be shown in the examples, direct storage at ambient temperatures (i.e. 18-22 °C) results in an unacceptable stability of the prepared composition.

Preferably, the separate components used in a method of the invention, are mixed with a stirring device such that the components form an emulsion. In a preferred embodiment, the invention provides a method for improving the stability of an egg, fat and sugar composition comprising adding a phospholipase to said composition, wherein said composition is an emulsion. The invention thus also provides a method for improving the stability of an egg, fat and sugar emulsion comprising adding a phospholipase to or incorporating a phospholipase into said emulsion. Preferably, said emulsion is a water-in-oil emulsion.

The composition or emulsion can for example be prepared by incubating an egg (for example a whole egg or egg yolk) with a phospholipase. In another aspect, the invention therefore provides a method for obtaining an egg, fat and sugar composition comprising incubating said egg with a phospholipase, preferably at 4 to 55 degrees
Celsius for 1 hour to 4 days, more preferably at 45 to 55 degrees Celsius for 2 to 4 hours and subsequently mixing the obtained treated egg with sugar and fat.

The above described definitions for the terms "egg", "fat" and "sugar" are applicable to this part of the invention as well.

As described herein within the experimental part, the phospholipase can also be used on a mixture of sugar and egg. The invention thus also provides a method for obtaining an egg, fat and sugar composition comprising incubating said egg and said sugar with a phospholipase, preferably at 4 to 55 degrees Celsius for 1 hour to 4 days, more preferably at 45 to 55 degrees Celsius for 2 to 4 hours and subsequently mixing the obtained treated egg and sugar with fat or alternatively worded, a method wherein said egg is premixed with said sugar, treating the obtained egg/sugar with a phospholipase and subsequently mixing the obtained treated egg/sugar with fat (for example butter (oil)).

Mixing of the components can be performed by using any suitable stirring device and for example in a range from 250 to 1250 rpm for a period of from 0.5 to 10 minutes and at a temperature ranging from 15 to 30 degrees Celsius.

The produced composition is preferably an emulsion, even more preferably a water-in-oil emulsion.

In yet another preferred embodiment, the invention provides a method for obtaining an egg, fat and sugar composition comprising incubating said egg or said egg and said sugar with a phospholipase, preferably at 4 to 55 degrees Celsius for 1 hour to 4 days, more preferably at 45 to 55 degrees Celsius for 2 to 4 hours and subsequently mixing the obtained treated egg with sugar and fat or the obtained treated egg and sugar with fat and wherein said method further comprises allowing the obtained egg, fat and sugar composition to stand at a temperature of 2-10°C for at least 24 hours. Alternatively worded, the invention further provides a method as described above, wherein the obtained composition is allowed to stand at a temperature of 2-10°C for at least 24 hours.

The term "for at least 24 hours" is defined above and also applies to this part of the invention.

The phospholipase in any of the above described methods can be any type of phospholipase (A, B or C). Preferably, said phospholipase is a phospholipase A.
All types of phospholipase A can be used in any of the above described methods, for example phospholipase A1 or phospholipase A2. Any type of phospholipase A1 can be used. Phospholipase A1 is wide-spread in nature, e.g. in microorganisms *E.coli*, in snake venoms, and in mammals in the brain, testis and liver. An example of a suitable commercially available phospholipase A1 is Lecitase Ultra™ (Novozymes). Any type of phospholipase A2 can be used. An example of a suitable commercially available phospholipase A2 is MaxaPal A2 (also known as Cakezyme™ (DSM)) or Lecitase L10 (Novozymes). A preferred phospholipase A2 is porcine pancreatic phospholipase A2 for example expressed in *Aspergillus niger* (MaxaPal A2 / Cakezyme™, DSM).

Another alternative is Panamore™(DSM) which is a lipolytic enzyme with a dual action on polar lipids, able to hydrolyse both the phospholipids into lyso-phospholipids and the galactolipids into galactomonoglycerides.

Yet another example is a PLA from Lipomod (Biocatalyst) or Lecitinase (Novozymes).

Any of the herein described methods can be performed with one type of phospholipase or with a combination of phospholipases. When a combination of phospholipases are used different phospholipases may be added at the same time (as separate enzymes or as a mixture) or the phospholipases are added after each other, for example, a second or third phospholipase is added after a first or second phospholipase has been allowed to incubate for a certain amount of time.

The invention thus also provides a method

- for improving the stability of an egg, fat and sugar composition comprising adding a phospholipase to said composition or
- for obtaining an egg, fat and sugar composition comprising incubating said egg with a phospholipase, preferably at 4 to 55 degrees Celsius for 1 hour to 4 days, more preferably at 45 to 55 degrees Celsius for 2 to 4 hours and subsequently mixing the obtained treated egg with sugar and fat,

wherein at least two different phospholipases are added.

The used phospholipase can be added as a liquid or as a solid formulation.

Preferably, the used phospholipase is added in an effective amount. The skilled person is capable of determining whether or not an enzyme amount is effective or not. Preferably, an effective phospholipase amount is in the range of 0.001 to 1 volume %. The amount needed is for example dependent on the specific phospholipase used.
More preferably said effective phospholipase amount is in the range of 0.01 to 0.1 % resulting in a PL conversion of from approximately 30 to approximately 100 percent respectively.

In one embodiment of the invention, the phospholipase can be used to pre-incubate the egg and optionally the sugar. The egg can be pre-incubated whole, alternatively only the egg yolk can be incubated. It has been found that it is advantageous to retain some lecithin in the egg for some applications. Therefore, in a preferred embodiment, the time the egg is incubated with the phospholipase (preferably phospholipase A) is limited to still retain some lecithin. Preferably between 10-70% of the lecithin present in the used eggs should be hydrolysed into lysolecithin. More preferably at least 20% lecithin should be hydrolysed and even more preferably at least 30%. In another preferred embodiment at most 60% lecithin should be hydrolysed and even more preferably at most 50% lecithin should be hydrolysed. Alternatively, incubated egg containing almost no remaining lecithin can be mixed with some non-incubated egg or some lecithin to obtain the desired quantities of lecithin and lysolecithin.

As an optional step in any of the herein described method, the produced composition comprising egg, sugar and fat can be further modified. Such a modification can be obtained in the composition itself or during baking (i.e. incorporated in the mixture but only effective during baking). For example, a further enzyme step can be introduced before, during or after phospholipase incubation. One can for example include a protease, (maltogenic) amylase, lipase or oxidase treatment. The amount of optional enzyme can be easily determined by the skilled person as well as the appropriate incubation conditions (such as temperature and time). Such an optional enzyme can be added as a liquid composition or as a solid (for example a powder formulated in flour) composition. Moreover, said optional enzyme can be encapsulated to regulate its release. As an example, the optional enzyme can be encapsulated in a fat which is solid a room temperature but liquefies at higher temperatures (such as 40 to 55 degrees Celsius). During baking an optional encapsulated enzyme will be released and can perform its activity.
In yet another embodiment, the invention provides a composition obtainable according to any one of the herein described methods:

- for improving the stability of an egg, fat and sugar composition comprising adding a phospholipase to said composition or
- for obtaining an egg, fat and sugar composition comprising incubating said egg with a phospholipase, preferably at 4 to 55 degrees Celsius for 1 hour to 4 days, more preferably at 45 to 55 degrees Celsius for 2 to 4 hours and subsequently mixing the obtained treated egg with sugar and fat.

A final composition can be presented in any form, such as a fluid, as a paste, as a brick or even as a dried substance. The final appearance is for example dependent on the used storage temperature or on subsequent performed steps such as drying. Furthermore, dilutions of a composition according to the invention are included as well.

Surprisingly a composition according to the invention performs very well in baking experiments, even better than when compared to the ingredients added as such).

In a further aspect, the invention provides a composition comprising egg, sugar, fat and a phospholipase. The terms egg, sugar, fat and phospholipase have been explained above. Additional ingredients in such a composition can be baking powder, salt, water, flavors and/or an emulsifier. Other ingredients are enzymes, such as a lipase, oxidase, protease or (maltogenic) amylase. A maltogenic amylase can for example be used to (further) improve the shelf life of a baked product prepared from a composition as described herein. The components egg, sugar and fat (preferably butter oil) are preferably present in approximately equal amounts. The amount of additional ingredient can be established by the skilled person.

Other examples of optional ingredients are Etenia (will be discussed in more detail later on), whey protein or any other starch component.

A composition according to the invention is preferably viscous, such that the composition can readily be poured and/or pumped, the composition at < 10°C is spoonable i.e. semi solid. Alternatively worded, a composition according to the invention is preferably spoonable, i.e. is semi-solid.

An advantage of a composition according to the invention is its shelf life which is improved compared to a combination of the ingredients without phospholipase. The
shelf life is especially improved due to an improved microbial stability. This was a very surprising observation. The invention therefore also provides a method for reducing or preventing microbial spoilage in an egg, fat and sugar composition comprising adding a phospholipase to said composition.

In a preferred aspect a composition according to the invention also provides flour. The flour is preferably added after the composition of egg, sugar and fat is properly/sufficiently mixed. In case the flour can not be mixed, a composition according to the invention can be present in a kit together with a suitable amount of flour.

In yet another embodiment, the invention provides a method for preparing a baked product, comprising adding flour to a composition as described above resulting in a batter and putting the obtained batter in a suitable baking mould and baking said product. When the flour has already been added, the invention provides a method for preparing a baked product, comprising putting the batter in a suitable baking mould and baking said product.

In a preferred embodiment, which can be applied to all aspects of the invention, additionally at least one of the components selected from the group consisting of calcium, yeast extract, modified starch, lipase, a protease, an oxidase, an amylase, a further phospholipase (being the same or different from the one used in the preparation of a composition as described herein), whey protein and/or amyloglucosidase is used in the production of a baked product, for example a cake. The cake can either be a regular cake, i.e. a cake comprising a regular amount of eggs and/or fat or a cake where eggs and/or fat have been reduced. The man skilled in the art knows which amount of eggs and/or fat is present in regular cakes, which amount will be dependent on the type of cake.

In a preferred embodiment of any one of the aspects of the invention also calcium is added to enhance the activity of the phospholipase A either at the pre-incubation or during the preparation of the batter to enhance the in-situ action of the phospholipase. In a preferred embodiment the calcium is added during preparation of the batter. It has been found especially advantageous to add approximately between 40 - 200 mg CaCl₂·H₂O per 5,000 CPU Phospholipase A (hereafter indicated as PLA) to the cake recipe. Preferably, between 50 and 150 mg CaCl₂·H₂O per 5,000 CPU PLA
is added to the cake recipe and most preferably at least 90 mg CaCl₂•H₂O per 5,000 CPU PLA. CPU (Chromogenic Phospholipase Unit = 1 EYU (Egg Yolk Unit)) is defined as the amount of enzyme that liberates 1 µmol of acid per minute from egg yolk at 40°C and pH8.0. Substrate in this method: rac 1,2-dioctanoyldithio phosphatidylcholine measured spectrophotometric at 405 nm.

The use of a phospholipase in a composition as described herein can further be used in the production of baked product (for example a cake) to improve at least one of the properties selected from the group consisting of: (i) batter viscosity, (ii) specific density, (iii) initial crumb softness, (iv) crumb pore homogeneity, (v) crumb pore diameter, (vi) crumb softness upon storage, (vii) shelf life and/or (viii) baked product volume.

The invention preferably provides a method for obtaining a baked product with at least one improved property selected from (i) baked product volume, (ii) initial crumb softness, (iii) shelf life and (iv) a less greasy taste, wherein said method comprises adding flour to a composition as described herein resulting in a batter and putting the obtained batter in a suitable baking mould and baking the product. If flour has already been added, the invention provides a method for obtaining a baked product with at least one improved property selected from (i) baked product volume, (ii) initial crumb softness, (iii) shelf life and (iv) a less greasy taste, wherein said method comprises putting the obtained batter in a suitable baking mould and baking the product.

Preferably, the other baked product properties are maintained. The term maintaining is hereby used to indicate that a property is maintained or improved.

Measuring whether a property is maintained, improved or deteriorated in general is measured by preparing a batter and/or a baked product (for example a cake) in an original recipe, not containing any phospholipase (for example phospholipase A) and another batter and/or baked product in a recipe containing phospholipase and optionally less eggs and/or fat and comparing a certain property. In case the properties of both are substantially the same, the property is maintained, in case they differ either an improvement or a deterioration has taken place. For all mentioned properties below a measurement method has been given as well as an indication when a property can be considered as improved.
The batter viscosity can be measured with a Farinograph by standard methods according to the International Association of Cereal Chemistry (ICC) and the American Association of Cereal Chemistry (AACC 54-2, ICC 115). The batter viscosity can also be determined with an Amylograph for example by using a Brookfield viscosity meter.

Whether the batter viscosity has improved or deteriorated can for example be measured by comparing the batter prepared with phospholipase, either containing or nor containing a reduced amount of eggs and/or fat, to a batter prepared without phospholipase. In case the batter viscosity is the same for both batters, it has been maintained. In case the batter viscosity has increased, it has improved.

The specific density can be measured by weighing a predetermined volume of batter. The specific density is improved if it is decreased.

The crumb softness of the baked product (for example a cake) is evaluated either empirically by the skilled test baker or measured by the use of a texture analyzer (e.g., TAXT2) as known in the art. Actually crumb firmness is measured as is known to the person skilled in the art. The crumb softness measured within 24 hours after baking is called initial crumb softness. The crumb softness more than 24 hours after baking is called crumb softness upon storage, and is also a measure for determining shelf life. In case the initial crumb softness has increased, it has improved. In case the crumb softness upon storage has increased, it has improved.

Crumb pore homogeneity can be evaluated empirically by the skilled test baker or by digital image analysis as known in the art (e.g. C-cell, Calibre Control International Ltd, Appleton, Warrington, UK). In case the deviation in pore size is small, the crumb is called more homogeneous. In case the deviation in pore size has become smaller, the property is improved.

Crumb pore diameter can be evaluated using digital image analysis as known in the art (e.g. C-cell, Calibre Control International Ltd, Appleton, Warrington, UK). In case the average crumb pore diameter decreases, the property is improved. Preferably, this is the case when at the same time the same (cake) volume is maintained.

The shelf-life of a baked product can be measured by determining the softness and/or the resilience in time of the baked product in time. This is part of the method to measure crumb softness, as is known to the person skilled in the art, whereby the relaxation of the baked product is also measured by the use of a texture analyzer (e.g., TAXT2) as known in the art.
The volume of a given baked product can be determined by an automated bread volume analyser (e.g. BVM-3, TexVol Instruments AB, Viken, Sweden), using ultrasound or laser detection as known in the art. In case the volume is increased, the property is improved. Alternatively the baked product height after baking in the same size tin is an indication of the baked product volume. In case the baked product height is increased, the baked product volume has increased.

The emulsion stability of the batter can be determined by determining the baked product height and visual analysis of the baked product structure. In case the baked product height has decreased, the emulsion stability of the batter has decreased. In case the baked product structure is more dense, the emulsion stability of the batter also has decreased.

Whether or not the taste of the baked product is less greasy is established by a taste panel. A properly trained test panel is capable of determining how the taste of a baked product should be rated.

In one embodiment of the invention a combination of at least two of the above-mentioned properties can be at least maintained when using phospholipase and optionally reducing the amount of eggs and/or fat used in the recipe or improved when using phospholipase, such as for example: batter viscosity and specific density; batter viscosity and initial crumb softness; batter viscosity and crumb pore homogeneity; batter viscosity and crumb pore diameter; batter viscosity and crumb softness upon storage; batter viscosity and shelf life of the baked product; batter viscosity and baked product volume; specific density and initial crumb softness; specific density and crumb pore homogeneity; specific density and crumb pore diameter; specific density and crumb softness after storage; specific density and shelf life of the baked product; specific density and baked product volume; initial crumb softness and crumb pore homogeneity; initial crumb softness and crumb pore diameter; initial crumb softness and crumb softness upon storage; initial crumb softness and shelf life of the baked product; initial crumb softness and baked product volume; crumb pore homogeneity and crumb pore diameter; crumb pore homogeneity and crumb softness upon storage; crumb pore homogeneity and shelf life of the baked product; crumb pore homogeneity and baked product volume; crumb pore diameter and crumb softness upon storage; crumb pore diameter and shelf life; crumb pore diameter and baked product volume;
crumb softness upon storage and shelf life; crumb softness upon storage and baked product volume; shelf life and baked product volume.

In another embodiment of the invention a combination of at least three of the above-mentioned properties can be at least maintained when using phospholipase and optionally reducing the amount of eggs and/or fat used in the recipe or improved when using phospholipase, such as for example: batter viscosity, specific density and initial crumb softness; batter viscosity, specific density and crumb pore homogeneity; batter viscosity, specific density and crumb pore diameter; batter viscosity, specific density and crumb softness after storage; batter viscosity, specific density and shelf life of the baked product, batter viscosity, specific density and baked product volume; specific density, initial crumb softness and crumb pore homogeneity; specific density, initial crumb softness and crumb pore diameter; specific density, initial crumb softness and crumb softness upon storage; specific density, initial crumb softness and shelf life of the baked product; specific density, initial crumb softness and baked product volume; initial crumb softness, crumb pore homogeneity and crumb pore diameter; initial crumb softness, crumb pore homogeneity and crumb softness upon storage; initial crumb softness, crumb pore homogeneity and shelf life; initial crumb softness, crumb pore homogeneity and baked product volume; crumb pore homogeneity, crumb pore diameter and crumb softness upon storage; crumb pore homogeneity, crumb pore diameter and shelf life; crumb pore homogeneity, crumb pore diameter and baked product volume; crumb pore diameter, crumb softness upon storage and shelf life; crumb pore diameter, crumb softness upon storage and baked product volume; crumb softness upon storage, shelf life and cake volume.

In addition also a combination of at least four of the above-mentioned properties can be at least maintained when using phospholipase and optionally reducing the amount of eggs and/or fat used in the recipe or improved when using phospholipase, such as for example: batter viscosity, specific density, initial crumb softness and crumb pore homogeneity; batter viscosity, specific density, initial crumb softness and crumb pore diameter; batter viscosity, specific density, initial crumb softness and crumb softness upon storage; batter viscosity, specific density, initial crumb softness and shelf life; batter viscosity, specific density, initial crumb softness and baked product volume; specific density, initial crumb softness, crumb pore homogeneity and crumb pore
diameter; specific density, initial crumb softness, crumb pore homogeneity and crumb softness upon storage; specific density, initial crumb softness, crumb pore homogeneity and shelf life; specific density, initial crumb softness, crumb pore homogeneity and baked product volume; initial crumb softness, crumb pore homogeneity, crumb pore diameter and crumb softness upon storage; initial crumb softness, crumb pore homogeneity, crumb pore diameter and shelf life; initial crumb softness, crumb pore homogeneity, crumb pore diameter and baked product volume; crumb pore homogeneity, crumb pore diameter, crumb softness upon storage and shelf life; crumb pore homogeneity, crumb pore diameter, crumb softness upon storage and baked product volume; crumb pore diameter, crumb softness upon storage, shelf life and baked product volume.

In another embodiment also a combination of at least five of the above-mentioned properties can be at least maintained when using phospholipase and optionally reducing the amount of eggs and/or fat used in the recipe or improved when using phospholipase, such as for example: batter viscosity, specific density, initial crumb softness, crumb pore homogeneity and crumb pore diameter; batter viscosity, specific density, initial crumb softness, crumb pore homogeneity and crumb softness upon storage; batter viscosity, specific density, initial crumb softness, crumb pore homogeneity and shelf life; batter viscosity, specific density, initial crumb softness, crumb pore homogeneity and baked product volume; specific density, initial crumb softness, crumb pore homogeneity, crumb pore diameter and crumb softness upon storage; specific density, initial crumb softness, crumb pore homogeneity, crumb pore diameter and shelf life; specific density, initial crumb softness, crumb pore homogeneity, crumb pore diameter and baked product volume; initial crumb softness, crumb pore homogeneity, crumb pore diameter and baked product volume; initial crumb softness, crumb pore homogeneity, crumb pore diameter and crumb softness upon storage and shelf life; initial crumb softness, crumb pore homogeneity, crumb pore diameter, crumb softness upon storage and baked product volume; crumb pore homogeneity, crumb pore diameter, crumb softness upon storage, shelf life and baked product volume.

In yet another embodiment also a combination of at least six of the above-mentioned properties can be at least maintained when using phospholipase and optionally reducing the amount of eggs and/or fat used in the recipe or improved when using phospholipase, such as for example: batter viscosity, specific density, initial crumb softness, crumb pore homogeneity, crumb pore diameter and crumb softness
upon storage; batter viscosity, specific density, initial crumb softness, crumb pore homogeneity, crumb pore diameter and shelf life; batter viscosity, specific density, initial crumb softness, crumb pore homogeneity, crumb pore diameter and baked product volume; specific density, initial crumb softness, crumb pore homogeneity, crumb pore diameter, crumb softness upon storage and shelf life; specific density, initial crumb softness, crumb pore homogeneity, crumb pore diameter, crumb softness upon storage and baked product volume; initial crumb softness, crumb pore homogeneity, crumb pore diameter, crumb softness upon storage, shelf life and baked product volume.

It has further been noticed by the inventors that the improved stability of an egg, fat and sugar composition as described above, provides a more equal distribution of chunks, such as chocolate chunks or raisins.

In one of its aspect, the invention provides a method for obtaining a baked product with at least one improved property selected from (i) cake volume, (ii) initial crumb softness, (iii) shelf life and (vi) a less greasy taste, wherein said method comprises adding flour to a composition as described herein resulting in a batter and putting the obtained batter in a suitable baking mould and baking the product, wherein said baked product is, for example, a cake, a biscuit, bread, brioche, muffin, cookies or doughnuts. In case the flour is already added to the composition, the adding of flour can be omitted. Other examples of baked products are a pastry, a pie, a tar or a quiche.

Typical ingredients of a cake are wheat flour, eggs and sugar. Optionally, baking powder, salt, water, emulsifiers (such as for example PGE's and monoglycerides), margarine, fat and/or oil are added (for example for pound cakes and muffins).

Also components to improve water binding such as hydrocolloids or modified starch can be used. In one embodiment of the invention, which can be applied to all the aspects of the invention, modified starch can be used to reduce the amount of fat used in the recipe. All types of modified starch can be used, for example modified potato starch, modified wheat or modified maize starch. Preferably modified potato starch is used, such as for example disclosed in US 6,864,063. Most preferably modified potato starch is used which is obtained by treating potato starch with amylomaltase, more preferably with amylomaltase derived from _Bacillus amyloliquefaciens_. An example of modified potato starch obtained by treating potato starch with amylomaltase derived
from *Bacillus amyloliquefaciens* is sold under the trademark Etenia (Avebe Food). It has been surprisingly found that in cakes comprising a reduced amount of fat, e.g. as low as 6 to 9 % w/w (i.e. a reduction of approximately 80 to 70 percent compared to a typical amount of 30% fat), and which are prepared using a combination of phospholipase and modified potato starch, desired cake properties as those mentioned above, e.g. batter viscosity, are improved if compared with cakes produced by using 30% w/w less fat and no addition of phospholipase and modified potato starch.

Optionally, flavouring agents such as vanilla extract, cocoa powder or yeast extracts can be added. An example of a suitable yeast extract is a yeast extract comprising at least 30% w/w 5’ ribonucleotides on the basis of sodium free dry matter.

In a preferred embodiment of the invention, which can be applied to all the aspects of the invention, a yeast extract is used which comprises at least 30% w/w 5’-ribonucleotides, preferably at least 34% w/w, 38% w/w, 40% w/w or 42% w/w, more preferably at least 44% w/w, 46% w/w, 48% w/w or at least 50% w/w 5’-ribonucleotides on the basis of sodium chloride free dry matter. It has been found that the use of such yeast extract not only improves the taste of the baked product (for example cake), but also has a surprising emulsifying effect, since upon its use, the viscosity of the batter improves.

In the context of the present invention, the phrase "5'-ribonucleotides" refers to the total amount of 5'-monophosphate ribonucleotides formed during RNA degradation, viz. 5'-monophosphate guanine (5'-GMP), 5'-monophosphate uracil (5'-UMP), 5'-monophosphate cytosine (5'-CMP), 5'-monophosphate adenine (5'-AMP), where 5'-AMP may be partially or completely converted into 5'-monophosphate inosine (5'-IMP). For example, in a yeast extract which comprises 30% w/w 5’-ribonucleotides on the basis of sodium chloride free dry matter, the total amount of 5'-GMP, 5'-UMP, 5'-CMP, 5'-AMP and 5'-IMP is 30% w/w on the basis of sodium chloride free dry matter.

In a preferred embodiment, a yeast extract is used wherein the total amount of 5’-GMP plus 5’-IMP is at least 15% w/w, preferably at least 17% w/w, 19% w/w, 20% w/w or 21% w/w, more preferably at least 22% w/w, 23% w/w, 24% w/w or 25% w/w, on the basis of sodium chloride free dry matter. Due to the constitution of RNA, from which the 5’-ribonucleotides arise, 5’-GMP and 5’-IMP will always be present in approximately equal amounts in this embodiment.
In the context of the present invention, weight percentage calculations of the 5'-ribonucleotides are based on the disodium salt heptahydrate thereof unless otherwise specified. All percentages are calculated on sodium chloride free dry matter. In the present invention, the phrase 'sodium chloride free dry matter' refers to the fact that for the calculation of the weight percentage the weight of any sodium chloride present is excluded from the composition. The measurement of sodium chloride in the composition and the above-mentioned calculation can be performed by methods known to those skilled in the art. An example of yeast extracts comprising 40% w/w 5'-ribonucleotides of which 20% w/w 5'-GMP plus 5'-IMP, weight percentages being based on sodium chloride free yeast extract dry matter, is sold under the trademark Maxarite® Delite (DSM Food Specialties, The Netherlands).

The yeast extract may be prepared by any method which yields a yeast extract which comprises at least 30% w/w 5'-ribonucleotides on the basis of sodium chloride free dry matter.

The yeast extract may be obtained by hydrolysis or autolysis. Methods to produce hydrolytic yeast extracts are known in the art see for example WO88/05267. In another embodiment, the yeast extract is obtained by autolysis, for instance as described in WO2005/067734.

It is possible to add additional enzymes to the baked product (for example cake) ingredients. Examples of such enzymes are amylolytic enzymes like fungal alpha-amylase, bacterial amylases, anti-staling amylases, amylglucosidases, lipolytic enzymes like lipases, galactolipases, proteolytic enzymes like endoproteases and exoproteases (carboxy- and aminopeptidases, redox enzymes (oxidases, etc.) and cross-linking enzymes (transglutaminase, etc).

In a preferred embodiment amyloglucosidase is added during the baked product production process. Amyloglucosidase has been found to have a positive effect on the batter viscosity and resulting in a finer crumb structure. Furthermore, the amyloglucosidase has a sweetening effect on the taste of the cake.

In another preferred embodiment, which can be applied to all aspects of the invention, another lipolytic enzyme, for example a lipase is added during the baked product production process in combination with Phospholipase. Surprisingly, it was found that adding an additional lipolytic enzyme increases the emulsion stability of the batter. Examples of suitable lipolytic enzymes are Bakezyme® L80,000 (a R. oryzae
lipase, available from DSM Food Specialties, The Netherlands) or Lipopan® 50 (a T. lanuginosis lipase, available from Novozymes, Denmark). An additional advantage is that this enables reduction of chemical emulsifier components, such as mono- and or diglycerides (E471) and polyglycerol esters of fatty acids (E475). The lipase can be added in a dosage between 0.5 - 5 wt % per kg of flour. In another aspect, the invention therefore relates to the use of a lipase in a baked product production for stabilizing the batter emulsion.

In one embodiment of the invention, which can be applied to all aspects of the invention, the phospholipase (preferably phospholipase A) and the optional additional ingredients are present in, for example, a cake mix. Cake mixes are often used at home because they are convenient. Most cake mixes simply require adding the package contents to eggs and oil in a bowl and mixing for two to three minutes. The mixture is then ready to be poured into a suitable baking mould and baked.

In a preferred embodiment of the invention, which can be applied to all aspects of the invention, additionally at least one of the compounds selected from the group consisting of calcium, yeast extract, modified starch, lipase and/or amyloglucosidase is used or added to the batter in combination with phospholipase. Also a combination of these compounds is possible, for example the addition of both calcium and yeast extract, the addition of both yeast extract and modified starch, the addition of both lipase and yeast extract, the addition of both amyloglucosidase and lipase, the addition of both lipase and modified starch, the addition of both modified starch and amyloglucosidase. In a preferred embodiment of the invention, which can be applied to all aspects of the invention, both yeast extract, according to the preferences indicated above, and modified starch according to the preferences indicated above is used or added to the batter or cake mix in combination with phospholipase. The yeast extract preferably comprises 30% w/w 5'-ribonucleotides on the basis of sodium chloride free yeast extract dry matter, preferably wherein the total amount of 5'-GMP plus 5'-IMP in the yeast extract is at least 15% w/w, preferably at least 17% w/w, 19% w/w, 20% w/w or 21% w/w, more preferably at least 22% w/w, 23% w/w, 24% w/w or 25% w/w, on the basis of sodium chloride free yeast extract dry matter. The modified starch is preferably modified potato starch, preferably a modified potato starch obtained by treating potato starch with amylomaltase derived from Bacillus amyloliquefaciens. It has been surprisingly found that cake containing 30% less fat, 20% less eggs, and a combination
of phospholipase, modified potato starch and a yeast extract comprising at least 30% w/w 5'-ribonucleotides on the basis of sodium chloride free dry matter, has very good quality in terms of volume, structure, mouthfeel and taste. This cake is very similar to the reference but containing much less calories per unit of weight.

In yet a further embodiment, the invention provides use of a composition as described herein for producing a baked product.

In another embodiment, the invention provides a baked product obtainable by any of the claimed methods or uses. Preferably, such a baked product is a cake.

The present invention covers all types of cake, including shortened cakes, such as for example pound cake and fat cake, and including foam cakes, such as for example meringues, sponge cake, biscuit cake, roulade, genoise and chiffon cake.

Sponge cake is a type of soft cake based on wheat flour, sugar, baking powder and eggs (and optionally baking powder). The only fat present is from the egg yolk, which is sometimes added separately from the white. It is often used as a base for other types of cakes and desserts. A basic sponge cake is made by beating the eggs with sugar until they are light and creamy, then carefully sieving and folding in the flour (which may be mixed with a small amount of baking powder, although the air incorporated into the egg mixture can be sufficient for a good rise). Sometimes, the yolks are beaten with the sugar first while the whites are beaten separately, to be mixed in later. The mixture is then poured into the chosen cake tin and baked. Before the mixture has cooled, after cooking, it is still flexible. This allows the creation of such varieties as the Swiss roll. This basic recipe is used for many treats and puddings, such as madeleines.

A pound cake is traditionally prepared of one pound each of flour, fat, eggs, and sugar, optionally complemented with baking powder.

In chiffon cake the butter/margarine has been replaced by oil. Sugar and egg yolk content has been decreased compared to pound or sponge cake and egg white content has been increased.

In cake recipes eggs provide natural emulsifiers as well as egg protein. Egg protein is important for froth forming in the batter and for the cake cohesiveness. In cake recipes wherein the amount of eggs has been reduced, especially if reduced of at least 30% w/w, 40% w/w or 50% w/w, the loss of egg protein can (partially) be
compensated by the addition of other protein sources and/or hydrocolloids. Examples of protein sources are whey protein, soy protein, modified wheat protein, albumin, etcetera. Examples of hydrocolloids are guar gum, alginate, pectin, xanthan gum, etcetera. Therefore in one embodiment of the invention one or more protein sources and/or one or more hydrocolloids are used in the cake recipe to replace the protein content present in the eggs removed.

It has been surprisingly found that when the amount of eggs in the cake is e.g. reduced up to 50% w/w and one or more protein sources and/or one or more hydrocolloids are added to replace the egg protein, cakes can be obtained wherein desired cake properties are at least maintained.

The egg volume can (partially) be replaced by use of water. Preferably (part of) the water content of the eggs may be replaced by water. Usually an egg contains about 75% water. The amount of water used in the recipe to replace the eggs may be at least 50% of the water content of the eggs removed. More preferably at least 60% of the water content of the eggs is replaced by water, even more preferably at least 75% and most preferably 100% of the water content of the eggs removed is replaced by water. It has surprisingly been shown that the water binding properties of the cake batter and cake are improved by the use of a phospholipase, enabling the use of more water in the cake recipe.

The invention is hereby illustrated with the following non-limiting examples.

**EXPERIMENTAL PART**

**Example 1**

A commercial cake mix comprising 'sugared whole egg' contains 50% whole egg mixed with 50% sugar. To prepare a more complete mix it was investigated how fat could be incorporated in sugared whole egg mix. Preferably, from those ingredients a stable emulsion has to be prepared to result in a more complete cake mix, a bakery convenient product.

Pound cake recipe is used for cake baking tests containing equal amounts of whole egg, sugar, butter and flour.

Sugared whole egg mix obtained commercially is mixed with liquid butter (oil) resulting in a cake mix containing, egg, sugar and liquid butter (oil) (as an example of a fat) in equal amounts.
Emulsions, 450 grams, were prepared with butter and standard or 0.1 % MAXAPAL treated sugared whole egg, using a turbine stirring device, as well known to the skilled person, at three rotations speeds, 500, 700 and 1000 rpm for 5 minutes at ambient temperature. The products were stored for 5 days at 4°C and afterwards at room temperature. Segregation was registered by photographs, taken with a Canon Powder shot camera.

The mixes were applied in cake baking, two emulsions with either standard or MAXAPAL treated egg, 2100 grams mix, were prepared. The emulsions were mixed for 10 minutes at 700 - 1000 rpm at room temperature.

For cake baking, sugared whole egg was incubated with 0.1 or 0.01 % MAXAPAL to show the differences in baked cakes as a function of the (egg) PL conversion, 100 and 50 % respectively. The conversion was not analyzed by P31 NMR.

Pound cake recipe was chosen to test the functionality of the emulsions. Table 1 presents the recipe.

Baking trials were performed with standard, MAXAPAL treated emulsions or addition of the separate ingredients. Cake volume and crumb softness were measured, photographs were taken for visual comparison.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount gram</th>
<th>Combined product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole egg</td>
<td>500</td>
<td>Sugared whole egg composition</td>
</tr>
<tr>
<td>Sugar</td>
<td>500</td>
<td>Sugared whole egg butter composition</td>
</tr>
<tr>
<td>Butter</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Flour</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Emulsifier</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Salt</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Baking powder</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>acidic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baking powder</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>bicarbonate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results

The sugared whole egg product was incubated with 0.1 % MAXAPAL for 3 hours at 50°C. Emulsions were prepared with standard or MAXAPAL treated egg and liquid butterfat as described above.

Emulsion stability

A clear difference is seen after mixing at 500 rpm the MAXAPAL treated emulsion compared to the standard product: the standard product shows segregation whereas the MAXAPAL treated emulsion does not or hardly show any segregation. Differences in the emulsions mixed at 700 or 1000 rpm were less pronounced.

Cake mix emulsions were stored for 5 days at 4°C followed by 2 weeks at ambient temperatures. MAXAPAL treatment of sugared whole egg, avoids egg segregation after storage as described of the cake mix emulsion using 500, 700 rpm or 1000 rpm. The amount of egg segregation in the standard emulsions decreased with increasing mixing speed.

Remarks:

- The viscosity of the 'sugared whole egg-liquid butter' emulsion was increased compared to the sugared egg dispersion as shown in Figure 1.
- Foam development was very limited during mixing even at 1000 rpm.

Cake baking 1

Sugared egg, 1400 gram, was incubated with 0.1 or 0.01 % MAXAPAL for 3 hours at 50°C. After cooling to room temperature, 700 gram liquid butter was mixed in the egg dispersion. After cooled storage overnight, cakes were baked applying those emulsions, the MAXAPAL treated egg or the standard.

Pound cakes were baked according the recipe mentioned in Table 1. The cake with the standard emulsion (i.e. not treated with MAXAPAL), was coarse and greasy. In the cake using Maxapal treated composition the crumb structure was finer compared to the cakes using the standard product of a DSM standard,
separately added liquid butter and non MAXAPAL treated sugared whole egg + liquid butter.

Cake baking 2

In a second cake experiment in which 0.1% and 0.01% MAXAPAL was tested differences in the crumb structures in cakes applying MAXAPAL 0.1 or 0.01 % were not observed.

It is assumed that the conversion of the egg phospholipids was (more than) 85 - 90 % applying 0.1 % MAXAPAL in sugared whole egg incubated for 3 hours at 50°C. Using 0.01 % MAXAPAL the PL conversion was approximately 50 %.

The volume and softness of the baked cakes were measured.

Table 2. Cake baking results referring to volume and crumb softness of 2 baking experiments

<table>
<thead>
<tr>
<th>mean specific cake volume ml/g</th>
<th>unit</th>
<th>Standard Pound cake</th>
<th>Sugared whole egg + separate butter</th>
<th>Emulsion standard</th>
<th>Emulsion + 0.1 % MXA</th>
<th>Emulsion + 0.01 % MXA</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean 100</td>
<td>%</td>
<td>100</td>
<td>105</td>
<td>100</td>
<td>122</td>
<td>116</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>mean Softness* 1 day</th>
<th>1246</th>
<th>662</th>
<th>774</th>
<th>559</th>
<th>524</th>
</tr>
</thead>
<tbody>
<tr>
<td>Softness* 7 days</td>
<td>gram</td>
<td>N.D.</td>
<td>1302</td>
<td>1420</td>
<td>929</td>
</tr>
<tr>
<td>Softness* 9 days</td>
<td>gram</td>
<td>2133</td>
<td>1244</td>
<td>1577</td>
<td>1267</td>
</tr>
</tbody>
</table>
softness is expressed in grams compression.
MXA = MAXAPAL
N.D. = not determined

- The volume of the cakes containing 0.1 or 0.01 % MAXAPAL treated sugared whole egg and liquid butter emulsion was significantly increased, 122 and 116 % respectively, compared to the standard cake or the sugared egg emulsion (+butter) both 100 %.

- After one day the crumb of the cakes applying 0.1 or 0.01 % MAXAPAL sugared whole egg and separate butter were softer, 559 and 524 grams compression, compared to the standard cake or the standard emulsion (+butter) in cake, 1246 and 774 grams respectively.

- The compression increased with factor 1.7 in 7 days for the standard and MAXAPAL containing cakes. After 9 days ambient storage, those factors were 1.7 and 2.2 for the standard mixes and MAXAPAL treated cake mixes.

In summary,

- From the sugared whole egg - butter mix an emulsion could be prepared by stirring the dispersion, using a Turbine stirring device applying 700 - 1000 rpm (rotations per minute) in 2 kg emulsion at room temperature.

The MAXAPAL treatment of sugared eggs results in the following benefits:

*In the emulsion*

- The emulsion containing 66 % sugared whole egg plus 33 % butter could be prepared after mixing with the turbine stirring device.

- The emulsions were stable for 2 weeks storage at room temperature.

*In the cakes*

- The volume of the cakes applying 0.1 % MAXAPAL treated sugared egg emulsion, was 122 % compared to the standard cake or the sugared egg emulsion, both 100 %.
The crumb of the cakes prepared with 'MAXAPAL treated cake emulsion' was softer compared to the standard cake (200 % compression) even softer applying the standard sugared egg emulsion (140 % compression).

Applying 0.01 % MAXAPAL resulted in a lower cake volume (116 %) and approximately the same crumb softness compared to 0.1 % MAXAPAL application.

The 'softness decrease' in time is equal for all products; as a consequence the cakes containing 'MAXAPAL treated cake emulsions' have a longer shelf life due to the initial softer cakes just after baking.

The emulsion preparation of the sugared egg + liquid butter resulted in
- An increased viscosity of the egg product / cake mix
- Some air incorporation (this was negligible at lab scale)

**Example 2**

- Sugar was mixed in whole egg (1:1)
- Maxapal was added and the whole was incubated at 50 °C
- Untreated samples were also included
- After cooling to 20-30 °C of the phospholipase treated composition, liquid butter was added
- The obtained samples were mixed at 500, 700 or 1000 rpm
- The samples were stored at ambient (18-22 °C) temperature
- These sample did not provide a satisfactory stability (i.e. these samples showed lack of segregation) (observed after 2 days of storage at ambient temperature)

**Example 3**

- Sugar was mixed in whole egg (1:1)
- Maxapal was added and the whole was incubated at 50 °C
- Untreated samples were also included
- After cooling to 20-30 °C of the phospholipase treated composition, liquid butter was added
- The obtained samples were mixed at 500, 700 or 1000 rpm
- The samples were stored at 4 °C
- After one week of storage at 4 °C, all Maxapal treated samples (i.e. 500, 700 and 1000 rpm) were stable.
The composition with non-treated egg, 500 and 700 rpm did not provide an acceptable stability. The non-treated 1000 rpm sample was at this point in time (one week storage) stable.

- After 2 weeks of storage all Maxapal treated samples were stable and the non-treated samples had segregated.
CLAIMS

1. A method for improving the stability of an egg, fat and sugar composition comprising adding a phospholipase to said composition.

2. A method according to claim 1, wherein said egg is pre-incubated with said phospholipase.

3. A method according to claim 2, wherein said egg and said sugar are pre-incubated with said phospholipase.

4. A method according to any one of claims 1 to 3, wherein said composition is an emulsion.

5. A method for obtaining an egg, fat and sugar composition comprising incubating said egg with a phospholipase, preferably for 1 hour to 4 days at 4 to 55 degrees Celsius, and subsequently mixing the obtained treated egg with sugar and fat, preferably butter and even more preferably butter oil.

6. A method according to claim 5, wherein said egg is premixed with said sugar, incubating said mixed egg and sugar with a phospholipase, preferably for 1 hour to 4 days at 4 to 55 degrees Celsius, and subsequently mixing the obtained treated egg/sugar with fat, preferably butter and even more preferably butter oil.

7. A method according to any one of claims 1 to 6, wherein said egg or said egg and said sugar are incubated with said phospholipase, adding fat and sugar to said phospholipase treated egg or adding fat to said phospholipase treated egg-sugar and wherein said method further comprises allowing the obtained egg, fat and sugar composition to stand at a temperature of 2-10°C for at least 24 hours.

8. A method according to any one of claims 1 to 7, wherein said egg is a whole egg or egg yolk.

9. A method according to any one of claims 1 to 8, wherein said phospholipase is a phospholipase A.

10. A composition obtainable according to any one of the methods of claims 1 to 9.

11. A composition comprising egg, sugar, fat and a phospholipase.
12. A composition according to claim 11, wherein said egg, sugar and fat are present in approximately equal amounts.

13. A method for preparing a baked product, comprising adding flour to a composition of any one of claims 10 to 12 resulting in a batter and putting the obtained batter in a suitable baking mould and baking said product.

14. A method for obtaining a baked product with at least one improved property selected from (i) cake volume, (ii) initial crumb softness and (iii) shelf life, wherein said method comprises adding flour to a composition according to any one of claims 10 to 12 resulting in a batter and putting the obtained batter in a suitable baking mould and baking the product.

15. A method according to claim 13 or 14 wherein said baked product is a cake, a biscuit, a bread, a brioche, a muffin, a cookie or a doughnut.

16. Use of a composition according to any one of claims 10 to 12 for producing a baked product.

17. A baked product obtainable by the method of any one of claims 13 to 15 or by the use of claim 15.

18. A baked product according to claim 17, which is a cake.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

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)

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

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

EPO-Internal , WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Category*</th>
<th>Citation of document, with indication and where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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<td>EP 1 900 282 A (PURATOS NV [BE])</td>
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<td>page 4, line 10 - line 56; example 1</td>
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<td>WO 2008/092907 A (DSM IP ASSETS BV [NL]; MASTENBROEK JOSE [NL]; HILDE JAN DIRK RENE [NL]) 7 August 2008 (2008-08-07)</td>
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X Further documents are listed in the continuation of Box C

X See patent family annex

* Special categories of cited documents

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'A' document member of the same patent family

Date of the actual completion of the international search

20 July 2010

Date of mailing of the international search report

26/07/2010

Name and mailing address of the ISA/Authorized officer

European Patent Office P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Fax (+31-70) 340-3016

Shadid, Rani a
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
</table>
the whole document  
page 3, paragraph 29 - paragraph 35  
page 5, paragraph 91 - page 6, paragraph 98  | 1-18 |
| X        | EP 0 319 064 A2 (UNILEVER NV [NL]; UNILEVER PLC [GB])  
7 June 1989 (1989-06-07)  
page 5; example 11  | 1-11 |
21 October 2003 (2003-10-21)  
column 4, line 19 - line 59; examples 1-3;  
table 1  
column 2, line 48 - line 50  | 3, 6 |
the whole document  | 1-18 |
the whole document  | 1-18 |
| A        | US 4 612 197 A (POSTNER HERMANN [DE])  
16 September 1986 (1986-09-16)  
the whole document  | 1-18 |
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
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</thead>
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<tr>
<td>EP 0426211</td>
<td>08-05-1991</td>
<td>AT 97555 T</td>
<td>15-12-1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 640167 B2</td>
<td>19-08-1993</td>
</tr>
<tr>
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<td></td>
<td>CA 2026447 A</td>
<td>30-03-1991</td>
</tr>
<tr>
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<td></td>
<td>DE 69004782 DI</td>
<td>05-01-1994</td>
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<tr>
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<td></td>
<td>DE 69004782 T2</td>
<td>17-03-1994</td>
</tr>
<tr>
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<td></td>
<td>DK 0426211 T3</td>
<td>31-01-1994</td>
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<tr>
<td></td>
<td></td>
<td>ES 2047827 T3</td>
<td>01-03-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 3244348 A</td>
<td>31-10-1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 7053083 B</td>
<td>07-06-1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MX 173217 B</td>
<td>09-02-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NZ 235428 A</td>
<td>25-06-1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TR 25502 A</td>
<td>01-05-1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5082674 A</td>
<td>21-01-1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZW 14890 A</td>
<td>13-02-1991</td>
</tr>
<tr>
<td>EP 1900282</td>
<td>19-03-2008</td>
<td>AT 473632 T</td>
<td>15-07-2010</td>
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<tr>
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<td>AU 2007291378 A</td>
<td>06-03-2008</td>
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<td></td>
<td>CA 2659318 A</td>
<td>06-03-2008</td>
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<td></td>
<td>CN 101573037 A</td>
<td>04-11-2009</td>
</tr>
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<td></td>
<td>EA 200900256 A</td>
<td>28-08-2009</td>
</tr>
<tr>
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<td></td>
<td>WO 2008025674 A</td>
<td>06-03-2008</td>
</tr>
<tr>
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<td>JP 201051195 T</td>
<td>21-01-2010</td>
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<tr>
<td></td>
<td></td>
<td>US 2010062105 A</td>
<td>11-03-2010</td>
</tr>
<tr>
<td>WO 2008092907</td>
<td>07-08-2008</td>
<td>AR 065148 A</td>
<td>20-05-2009</td>
</tr>
<tr>
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<td></td>
<td>AU 200829726 A</td>
<td>07-08-2008</td>
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<tr>
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<td></td>
<td>CA 2676707 A</td>
<td>07-08-2008</td>
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<td></td>
<td>CL 3362008 A</td>
<td>14-03-2009</td>
</tr>
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<td>EA 200901055 A</td>
<td>30-12-2009</td>
</tr>
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<td></td>
<td>EP 2124581 A2</td>
<td>02-12-2009</td>
</tr>
<tr>
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<td>JP 2010517521 T</td>
<td>27-05-2010</td>
</tr>
<tr>
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<td></td>
<td>US 2010062106 A</td>
<td>11-03-2010</td>
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<tr>
<td>US 2004076717</td>
<td>A1 22-04-2004</td>
<td>NONE</td>
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</tr>
<tr>
<td>EP 0319064</td>
<td>07-06-1989</td>
<td>AU 2638988 A</td>
<td>08-06-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 1310224 C</td>
<td>17-11-1992</td>
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<tr>
<td></td>
<td></td>
<td>DE 3871095 DI</td>
<td>17-06-1992</td>
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<td></td>
<td>ES 2032000 T3</td>
<td>01-01-1993</td>
</tr>
<tr>
<td></td>
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<td>GR 3005049 T3</td>
<td>24-05-1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 1199559 A</td>
<td>10-08-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 1858349 C</td>
<td>27-07-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 5081228 B</td>
<td>11-11-1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PH 25356 A</td>
<td>13-05-1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5028447 A</td>
<td>02-07-1991</td>
</tr>
<tr>
<td>US 6635777</td>
<td>BI 21-10-2003</td>
<td>AT 260049 T</td>
<td>15-03-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BR 0012257 A</td>
<td>12-03-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2371881 A</td>
<td>28-12-2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 1355676 A</td>
<td>26-06-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 60008529 DI</td>
<td>01-04-2004</td>
</tr>
<tr>
<td></td>
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<td>DE 60008529 T2</td>
<td>23-12-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1185179 A2</td>
<td>13-03-2002</td>
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<td></td>
<td></td>
<td>ES 2213019 T3</td>
<td>16-08-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 0078162 A2</td>
<td>28-12-2000</td>
</tr>
<tr>
<td></td>
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<td>JP 3589904 B</td>
<td>17-11-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2001000138 A</td>
<td>09-01-2001</td>
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<td>Patent family member(s)</td>
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</tr>
<tr>
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<tr>
<td>US 2001055635 A1</td>
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<td>NONE</td>
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<tr>
<td>WO 03097825 A</td>
<td>27-11-2003</td>
<td>AU 2003232821 A1</td>
<td>02-12-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BR 0311205 A</td>
<td>15-03-2005</td>
</tr>
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<td></td>
<td>CA 2486529 A1</td>
<td>27-11-2003</td>
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<tr>
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<td>CN 1659277 A</td>
<td>24-08-2005</td>
</tr>
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<td></td>
<td></td>
<td>CN 101684470 A</td>
<td>31-03-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1506291 A2</td>
<td>16-02-2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2005525818 T</td>
<td>02-09-2005</td>
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<tr>
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<td></td>
<td>JP 2009183302 A</td>
<td>20-08-2009</td>
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<tr>
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<td></td>
<td>US 2010047877 A1</td>
<td>25-02-2010</td>
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<tr>
<td></td>
<td></td>
<td>US 2007207521 A1</td>
<td>06-09-2007</td>
</tr>
<tr>
<td>US 4612197 A</td>
<td>16-09-1986</td>
<td>CA 1233693 A1</td>
<td>08-03-1988</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 3423699 C1</td>
<td>16-01-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0166284 A2</td>
<td>02-01-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ES 8608296 A1</td>
<td>01-12-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 80711 A</td>
<td>01-07-1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 8504431 A</td>
<td>26-02-1986</td>
</tr>
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